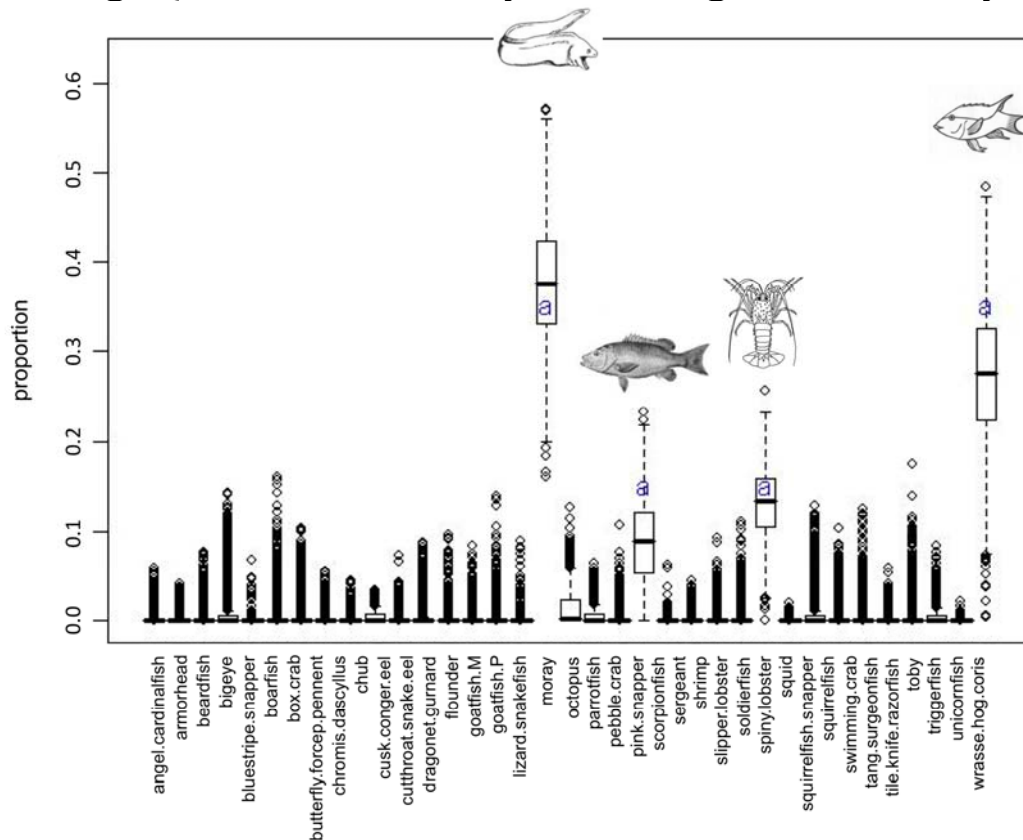# Hawaiian Monk Seals and Their Prey: Assessing Characteristics of Prey Species Fatty Acid Signatures and Consequences for Estimating Monk Seal Diets Using Quantitative Fatty Acid Signature Analysis

Sara Iverson, Jacinthe Piché and Wade Blanchard

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Hawaiian Monk Seals and Their Prey: Assessing Characteristics of Prey Species Fatty Acid Signatures and Consequences for Estimating Monk Seal Diets Using Quantitative Fatty Acid Signature Analysis

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PREFACE

This report has been sponsored by the Pacific Islands Fisheries Science Center in collaboration with Dalhousie University and provides results of research efforts to ascertain the feeding habits of the Hawaiian monk seal throughout its current range. This research focuses on the use of fatty acid signature analysis to determine the occurrence and composition of a wide variety of prey species consumed by monk seals. Subsequent publication of these data and additional analysis will address the most salient aspects of this work as it relates to the foraging ecology of the Hawaiian monk seal.

This report was funded by contract AB133F-03-SE-1195. Because the report was prepared by an independent investigator, its statements, findings, conclusions, and recommendations do not necessarily reflect the official views of the National Marine Fisheries Service, NOAA, U.S. Department of Commerce.

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EXECUTIVE SUMMARY

The Hawaiian monk seal (*Monachus schauinslandi*) was listed as endangered under the US Endangered Species Act (ESA) in 1976. The species is now estimated to number around 1200 to 1300 seals and declining. Seals live principally at six colonies in the Northwestern Hawaiian Islands (NWHI), but with small and increasing numbers in the main Hawaiian Islands (MHI). In addition to small population size, several natural and anthropogenic factors substantially affect the vitality and likely the persistence of the species, including entanglement in marine debris, predation by sharks, infectious disease, and breeding habitat erosion and disappearance as sea level rises. Moreover, limited availability of food (and perhaps inter- and intra-specific competition for food resources) appears to be an important constraint on growth, survival, recruitment of juveniles, and, consequently, abundance of seals in the NWHI.

Information about the diversity and predominance of prey of marine mammals has mostly been obtained from recovery of hard parts of prey from feces and stomach contents. The methods are known to have numerous potential biases, owing to differential rates of digestion and passage of various structures of different fishes and invertebrates, and diet estimates derived represent only the last meal(s) near haulout sites. Analyses of dietary fatty acids (FAs) have helped resolve some of these biases for several marine mammals and seabird species. A project was begun in 1998 to determine whether quantitative FA signature analysis (QFASA) could be used to better characterize the diet of monk seals and perhaps resolve the biases in use of hard parts of prey. This report represents the culmination of that work to elucidate foraging habits of monk seals in the NWHI and MHI.

The development of the QFASA method for monk seals was comprised of multiple steps, including using FA signatures of monk seals alone to qualitatively characterize aspects of their foraging, analysis of the prey FA database and issue relating to estimating diets. The use of QFASA simulations were explored to further understand the reliability and degree to which prey species can be distinguished based on their FA signatures. Investigations then explored the best way to approach the use of QFASA in modeling diets of free-ranging monk seals and, finally, diets were estimated for all free-ranging monk seals that have been sampled.

A total of 248 monk seal blubber samples from throughout the NWHI and MHI were analyzed. Although sampling did not permit a thorough analysis of each demographic group and atoll, preliminary analyses revealed some differences in FA profiles among subsets of demographic groups and general regions. Large variability was apparent among individual monk seals and we detected differences in diets between juveniles and adults, with differences between sexes being less pronounced. Differences in diets among major regions are consistent with known differences in prey-species assemblages between MHI and NWHI. Quantitative modeling of monk seal diets using QFASA was the next step in shedding light on these qualitative variations and differences found among monk seal diets.

Given the extraordinarily large number of prey species in this ecosystem, which presents both ecological and mathematical problems, we concluded upon a method of grouping prey species using a combination of previous methods, but with emphasis on grouping taxa in the context of

ecological relationships. The general ecological and diet guild of each species was categorized as one of five types: 1) benthic herbivores (browser, grazers), 2) planktivores (algal planktivores, zooplanktivores, gelatinous plankton), 3) benthic invertebrate feeders (corallivore, sessile invertebrates such as sponge, mobile invertebrates), 4) piscivores, or 5) detritivores/omnivores, based on data available from the literature. Crustaceans and cephalopds were also given special consideration. These groupings were then evaluated using a complex series of simulated diet estimations. These simulations aimed to basically push the limits of QFASA, by testing complex diet mixtures specified in a pseudo predator using a large number of prey species groups (40). This was used as a further tool to evaluate the ability to estimate diet from FA signatures and to characterize among-species overlap in signatures, allowing identification of prey species that have the potential to be misclassified as one another and, therefore, allowing a deeper understanding of model diet estimates. Simulations demonstrated that the QFASA model was able to reasonably estimate diet, but it was necessary to be aware of several important issues: 1) that of overlap in some problematic species, as well as 2) noise in terms of "false positives" (groups appearing in estimated diets but not specified in the pseudo diet).

Across all locations sampled in the NWHI and MHI, monk seal diets were estimated to be comprised of a mixture of species, the most abundant of which were boarfish, duckbill, box crab, flower snapper, shrimp, squid, squirrelfish snapper, and tang/surgeonfish. Overall, estimated diets of monk seals during the period of this study (1998-2002) were dominated in large part by deep-slope species, consistent with recent results from seal dive and location analyses, as well as from animal-borne video. However, there was substantial variation in diet among individuals, demographic groups (especially between juveniles and adults/subadults) and locations, as evidenced by the complementary results of both discriminant analyses and QFASA diet estimates. In repeat-sampled individuals, estimated diets tended to be similar within individuals; the largest differences that were found within an individual were particularly evident when a seal was sampled as a juvenile and then later as a subadult.

In terms of diet estimates, there remain some issues that require caution in interpretation. Firstly, it should be acknowledged that diet estimates may represent some overlap among certain prey groups. Additionally, the absence of some prey species in diets may also be puzzling: although lobsters, eels and wrasses were well-simulated and well-differentiated, other than for some morays they did not appear in estimated seal diets. It is possible that earlier diet estimates based on scats and spews, which identified these as diet items, were entirely biased to nearshore feeding and resistant hard parts (or that spews of eels represent "non-intake" of those prey), rather than the main prey that dominate the diet from primarily deepwater feeding. It is also possible that these prey could be, in part, mistaken for other prey that were identified. A third alternative is that monk seals switched primary prey intake from that documented during earlier studies.

Thus, some questions remain in interpreting the findings of these analyses. We completed all analyses possible with the resources available, but there remain questions that could be addressed in the future. We are confident that we have sampled most of the important species to monk seals, but this has not been an easy task – for some species, collection of even a few individuals has required incredible effort. Nevertheless, some species were not able to be obtained at all. For instance, one of the most abundant subphotic species is a small snapper that could not be

obtained. Thus, whether its signature overlaps with that of other deepwater snappers is as yet unknown. An investigation of how FA signatures of prey vary among habitats, across food webs and trophic levels, and within multispecies foraging aggregations would provide important insight into how we model seals and whether we should use prey within only certain regions. Further work on QFASA simulations could provide insight into the mathematical issues that arise when too many species are specified in the pseudo diet and how this relates to the actual modeling of the predator. Diet estimates could be further elucidated through additional series of analyses, such as sequential leave-one out modeling, or further analyses of prey groups and grouping procedures. These are currently beyond the scope of the current work, but could be considered.

Addendum to Report:
Following completion of this final report, further work has been conducted to refine analyses and prepare subsections for publication in the primary literature. In our initial work presented here, an in-depth evaluation of the prey FA database (Sections III and IV), targeting species of interest in terms of monk seal diet, was conducted in parallel with monk seal diet estimations. These studies were aimed at using FAs to further our understanding of the ecology of key forage fish and invertebrates through the detection of specific FA patterns and their variation in the prey species assemblage as a preface to modeling monk seal diets. Because of the constraints associated with QFASA in diet modeling, it was originally necessary to reduce the number of species groups to be incorporated in diet estimations by creating the lowest possible number ($n = 40$) commensurate with the number of FAs used in modeling. However, these restrictions were not necessary when investigating prey alone – that is, to best explore the ecological patterns of fish and invertebrates in the NWHI. Thus, a larger number of species groups was created ($n = 47$), which allowed some further breakdown of groups by taxonomy and diet. This substantially increased the resolution of analyses, and allowed for new FA patterns to emerge. A summary contrasting the findings of the work in this report (Section III) versus the more in depth prey FA investigations by Piché et al (*in review*) is presented in Appendix 3. Discriminant function analysis (DFA) performed on the 47 groups revealed that groups with similar FA signatures associated into five major functional groups: herbivores, planktivores, carnivores (including piscivores, benthic carnivores, and omnivores/detritivores), crustaceans, and cephalopods. Perhaps more interestingly, DFAs performed on all groups, as well as within each functional groups revealed segregation of species groups along a depth gradient: deepwater species (subphotic and slope) were more closely associated, and clearly separated from species thriving on shallow reefs. To further assess the new species groupings in the QFASA model, prey simulation trials were then conducted using the 47 groups following the same processes described and applied in this report (Section IV). The simulation results were found to be almost identical (Piché et al., in press). Further work will be aimed at pursuing prey FA investigations at the species level, to explore yet more in-depth patterns among individual species.

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CONTENTS

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I. INTRODUCTION, BACKGROUND AND STRATEGIC APPROACH

Understanding the foraging ecology of Hawaiian monk seals (*Monachus schauinslandi*) and the prey species upon which they depend has been a central issue in assessing management and recovery plans. Given the well-known biases and limitations of methods to assess pinniped diets from collections of spews or from recovering hard parts from feces, our aim has been to determine whether aspects of monk seal foraging could be inferred qualitatively using fatty acid (FA) signatures of blubber stores and whether monk seal diets could be estimated using quantitative FA signature analysis (QFASA; Iverson et al., 2004).

FAs are the main constituent of most lipids and unlike other nutrients, such as proteins that are readily broken down during digestion, FAs are released from ingested lipid molecules (e.g., triacylglycerols) during digestion, but are not degraded. The FAs of carbon chain-length 14 or greater pass into the circulation intact and are generally taken up by tissues the same way. Since a relatively limited number of FAs can be biosynthesized by animals (Cook, 1991), it is possible to distinguish dietary versus non-dietary components for certain FAs. Once taken up by tissues, FAs are either used for energy or re-esterified, primarily to triacylglycerols, and stored in adipose tissue. Although the metabolism of FAs appears to vary somewhat depending on predator species and likely on other factors such as diet/physiological state, such that the composition of predator tissue will not exactly match that of their prey, many FAs are deposited in adipose tissue with little modification and in a predictable way. FAs in marine organisms are extremely diverse, and numerous studies have demonstrated that specific FA patterns are passed from prey to predator both near the bottom and top of food webs, allowing qualitative inferences into trophic relationships (reviewed in Dalsgarrd et al., 2003; Iverson et al., 2004; Budge et al., 2006; Iverson, 2009).

More recently, a statistical model (QFASA) has been developed which allows the quantitative estimation of predator diets from the FA signatures of their lipid stores and a comprehensive prey database. The performance of QFASA has been validated in several northern pinniped species, mink (*Mustela vison*), polar bears (*Ursus maritimus*) and seabirds in the Bering Sea (e.g., Iverson et al., 2004, 2006, 2007; Nordstrom et al., 2008; Wang et al., 2010). There are a number of requirements in using QFASA, including appropriate sampling and analysis of predator tissue, an accounting for predator metabolism effects on FA deposition, and a comprehensive prey database and evaluation of the extent to which prey species can be characterized and distinguished by their FA signatures. The latter two requirements require careful consideration in the context of the current work on monk seals and their prey, and are discussed below.

This report represents the culmination of work that was begun in 1998 to examine the use of FA signatures and QFASA to elucidate foraging habits of monk seals in the Northwestern and main Hawaiian Islands (NWHI and MHI, respectively). In order to clearly present the issues that have needed to be addressed in this work and to set the final analyses into context, we first review and summarize the interpretation and results of using FA signatures of monk seals alone to qualitatively characterize aspects of their foraging. We then present the detailed and sequential analyses of the prey database and the use of QFASA simulations explored to understand the reliability and degree to which we can distinguish prey species based on their FA signatures.

Based on these analyses, we make conclusions about the best way to approach the use of QFASA in modeling diets of free-ranging monk seals. And finally, we present the current estimations of diets for all free-ranging monk seals that have been sampled.

Understanding and Accounting for Lipid Metabolism in Monk Seals

To understand and account for lipid metabolism and FA deposition in the predator, Iverson et al. (2004) proposed the use of "calibration coefficients" (CCs), which are simple weighting factors calculated from long-term diet studies. If an individual predator has been on a long-term diet of a constant FA composition, its major adipose tissue FA stores would maximally resemble this diet composition, and differences would be due to metabolic processing of individual FA. Based on a study of 10 captive monk seals held at SeaWorld, San Antonio, Texas (Appendix A), we were able to 1) verify that monk seal blubber FA signatures are predictably influenced by their prey (Fig. 1) and, therefore, that FAs can indeed be used to qualitatively understand aspects of foraging in this tropical species, and 2) estimate calibration coefficients for monk seals that were similar to those of other phocids (Fig. 2).
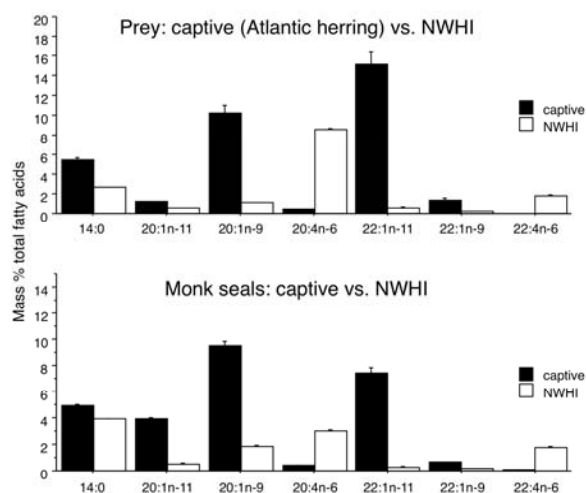
Figure 1.-- Selected dietary FAs (mean ± SE) in the (top panel) prey (herring) of captive monk seals in comparison to prey in the Northwestern Hawaiian Islands (NWHI) and (bottom panel) in captive monk seals ($n = 10$) fed Atlantic herring in comparison to that of the blubber of free-ranging monk seals ($n = 157$) in the NWHI. Values for captive prey are the average of all herring analyzed ($n = 25$, from 5 different lots fed) and for wild prey are simply the average of all prey species previously analyzed in the NWHI data base ($n = 1540$ individuals; S. J. Iverson and G. Antonelis, pers. comm.) for comparison purposes. The differences in the general FA signatures of the two types of prey (captive-fed Atlantic herring vs. prey in the NWHI) were clearly reflected in the blubber signatures of monk seals. The high levels of 14:0, 20:1n-11, 20:1n-9, 22:1n-11 and 22:1n-9 of Atlantic herring were reflected in the captive seals, while much lower levels of these components in wild prey were reflected in the wild seals. Wild prey in the NWHI in general contained much higher levels of 20:4n-6 and 22:4n-6, which were also found in higher levels in the wild seals in the NWHI. Clearly, monk seal blubber FA signatures are predictably influenced by those of their diet (Appendix A).

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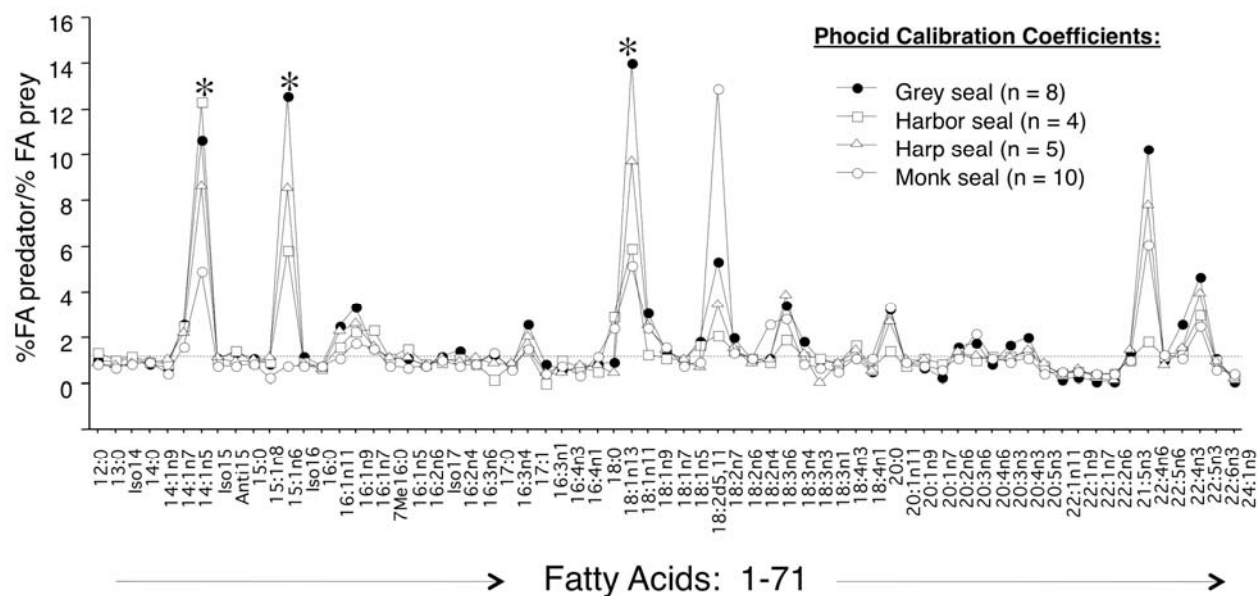
Figure 2.--Calibration coefficients (mean ± SE) calculated from controlled feeding experiments on monk seals in comparison to three other species of phocid seal [*reproduced from* Iverson (2009)]. CCs were estimated within each individual (note: in all cases the SE is too small to see) for all 71 FAs quantified according to Iverson et al. (2004). The 1:1 line denotes the deviation of a given FA in a predator from that consumed in its diet. *indicates examples of FAs with large deviations from 1:1 but which usually occur at minor or trace amounts (< 0.5%) in seals and their prey, which have contribution from biosynthesis in predators, and are routinely not used in QFASA modeling. Monk seals appear to be similar to other phocid seals in how they deposit and metabolize dietary FAs. Using these calibration coefficients, the diets of captive monk seals were generally well-estimated (see Appendix A).

Strategic Approach Undertaken

We used a series of approaches in this report to apply the use of FAs to understand the diets of free-ranging monk seals. We first examined monk seal FA signatures as qualitative indicators of diet patterns among individuals and demographic groups across the NWHI and MHI. We then conducted a series of analyses to examine the degree to which prey in the NWHI and MHI could be characterized by their FA signatures and to examine the performance of the QFASA model in differentiating these prey. Finally, we quantitatively estimated the diets of free-ranging monk seals using QFASA.

We used discriminant function analysis (DFA) to first explore multivariate analyses of prey FAs and we then used two routines of the QFASA modeling procedures (pseudo predator diet simulations and prey-on-prey simulations, Fig. 3) to further examine prey FA signatures. Given the complicated series of analyses used to thoroughly examine the prey FA database and FA subsets (Sections III - V), which was necessary prior to being able to model monk seal diets (Section VI), we present an overall "road map" to graphically summarize the approach we took (Fig. 4).

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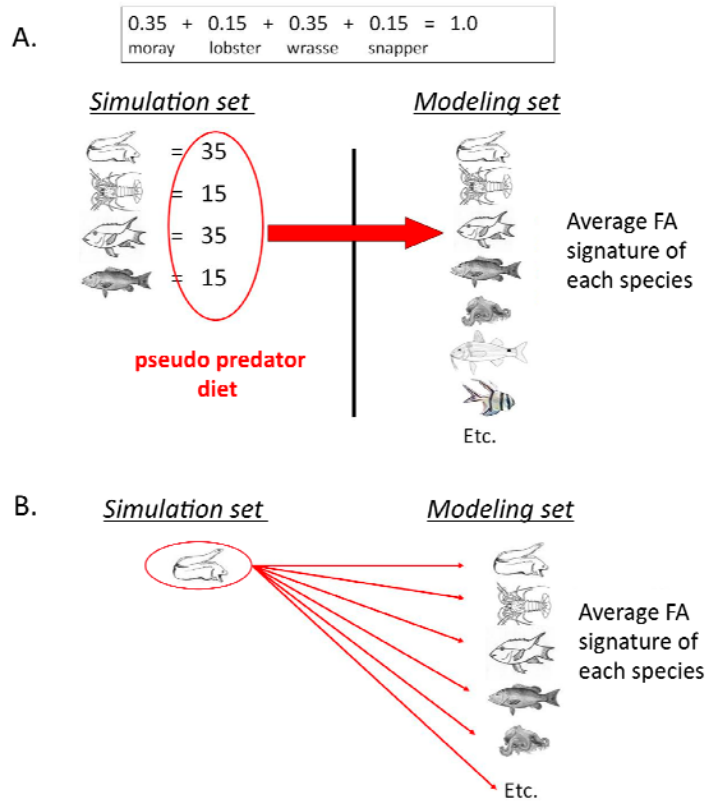
Figure 3.--Graphic depiction of QFASA simulation studies. **A. Pseudo predator diet simulations**. For each species group specified in the pseudo predator diet, the individuals sampled are randomly split into two sets: a simulation set and a modeling set. From the modeling set, a "pseudo predator" FA signature (hypothetical mixture of potential prey) is created. The proportion for each species group is specified (with all proportions positive and sum to 1). In this example the pseudo predator diet composition specified is 35% moray eel, 15% lobster, 35% wrasse, and 15% pink snapper. The pseudo predator FA signature is then modeled with the other half of the individuals of those prey and all other prey species in the library. The procedures of splitting the specified prey, creating the pseudo signature, and modeling that signature are each repeated 1000 times for each stipulated diet. This allows for overlap among species groups to be characterized; if there is no overlap, the simulation results will reflect the species group proportions specified in the pseudo diet. See Iverson et al. (2004) for further details. **B. Prey on prey simulations**. These simulations are performed to determine how well each prey species was likely to be identified as itself and differentiated from all other prey in the library using QFASA. The procedures for prey on prey are similar to the pseudo diet simulation procedures in A, but differ in that a single species is modeled essentially as a "predator". For each focal species group, the individuals are randomly split into two groups of equal sample size: a simulation group and a modeling group. An average FA signature of the simulation group is then modeled in QFASA on the FA signatures of the modeling group and all other prey species in the library. This process of splitting and modeling is repeated 1000 times. These simulations also allow for the direct identification of which prey species a given species might be mistaken for in diet estimations.

Using the tools outlined in Figure 3, the "road map" of our approach is presented in Figure 4.

III. The Prey Database for the NWHI

Can NWHI prey species be characterized on the basis of their FA signature?

100 potential monk seal prey species

⬇

40 groups of species based on taxonomical relatedness and ecology

DFA

Herbivores (n=5 groups)	Crustaceans (n=6 groups)	Carnivores (n=25 groups)
DFA	**DFA**	**DFA**
YES	YES*	YES *

** Problematic groups identified

IV. QFASA Prey Simulations

Do the 40 species groups perform well in QFASA?

1. **Initial set of simulations**: which mixture of species groups (pseudo predator diet) performs best in the model?

Trials 1 – 5	FA sets
Pseudo diets of 4 or 6 groups	Dietary (n = 31)
with 10% noise and without noise	Extended (n = 39)

Best pseudo diets: Trial 1 (n=4 groups) and Trial 3 (n=6 groups)

⬇

2. **Combining problematic groups**: some snappers from different groups misclassified for one another; similar issues for the two goatfish groups. Does combining problematic groups improve simulation results? (Trial 6 - 7) **NO** (better to keep groups separated)

⬇

3. **Evaluation of FA subsets**: which FA set yields best simulation results?

Pseudo Diets	FA sets	Noise
Trial 1	Dietary (n = 31)	10 %
Trial 3	Extended (n = 39)	None
	Modified 1 (n = 33)	
	Modified 2 (n = 34)	

Best results:

Effect of removing false positives: will removing false positive groups representing > 1% of the estimated diet improve the simulation results? **YES**

IV. QFASA Prey Simulations, cont'd.

Modeling 10 species of interest:

Can a pseudo diet composed of **10% of each of 10 species of interest** (armorhead, flounder, flower snapper, moray, octopus, spiny lobster, squid, squirrelfish snapper, tang/surgeonfish, and triggerfish) be accurately estimated?

YES

NO

Remove false positives and re-run simulation

NO

Split 10 species of interest into **2 sets of five species** and run these sets in two separate simulations

Remove false positive sequentially in each set, and re-run simulation after each exclusion

YES

4. Prey modeled on other prey: can the FA signature of individual prey groups be accurately differentiated from other prey groups? **YES**: majority of the groups estimated individually on all other groups were correctly identified as themselves; some problematic species identified.

V. Evaluation of FA Subsets and Calibration Coefficients (CCs) in estimating Monk Seal Diets using QFASA

Fairly consistent estimates of diets across all FA subsets and CC combinations; therefore all results can be averaged .

VI. Estimates of Monk Sea Diets using QFASA

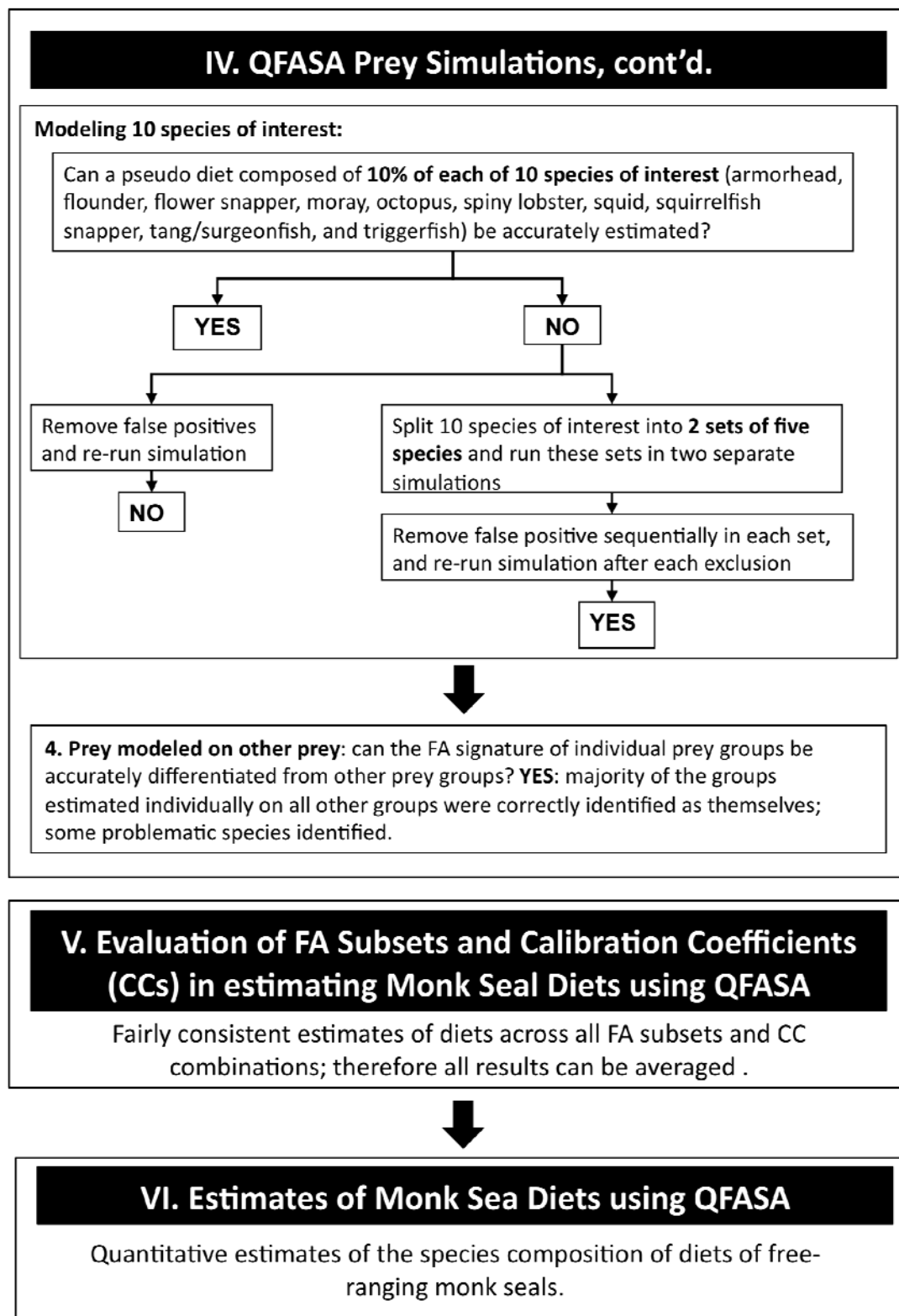Quantitative estimates of the species composition of diets of free-ranging monk seals.

Figure 4.--Graphic depiction of strategic approach used in prey FA analyses for subsequent QFASA modeling of monk seal diets. The headings III – VI refer to Sections III – VI of this report.

II. MONK SEAL FATTY ACIDS AS QUALITATIVE INDICATORS OF DIET

To date, we have analyzed blubber biopsies from a total of 248 monk seals throughout the NWHI and MHI (Table 1). Although the representation of each age class and sex does not permit a thorough analysis of each demographic group and atoll, preliminary analyses can be used to examine the qualitative variation and differences in monk seal diets among some subsets of demographic groups and general regions.

Table 1. Monk Seals Sampled - 1998-2002   **n = 248**

Age class	Kure	Midway	P & H	Lisianski	Laysan	FFS	Main
Ad F	4	8	1	4	6	6	3
Ad M	4	4	0	5	15	24	4
Sub F	0	5	0	3	1	15	0
Sub M	1	3	0	1	1	8	4
Juv F	5	2	3	2	11	26	1
Juv M	7	7	4	8	14	25	3

Discriminant function analyses (DFA, SPSS Inc., Chicago, IL) were performed according to Iverson et al. (2002), using a subset (as dictated by sample size) of FAs which had the largest overall variance and an overall mean of $\geq 0.4\%$ of total FAs. Percentage values for these FAs were transformed into log ratios prior to DFA by first renormalizing their values over 100% and then dividing the value for each of the FAs by the value for a reference FA to improve normality. The reference FA used was 18:0 because it is consistently found in all samples of seals and their prey and exhibits variability, meeting the requirements for transformation (Aitchison, 1986). The resulting ratios were then log transformed and used in the analyses. Since the log of zero cannot be taken, zero values for any of the FAs were changed to 0.005% prior to the calculation of the log ratio. This value of 0.005% was selected as a value that is below what we considered to be the minimum detectable level (0.01%) but which would not be so small as to result in extreme outliers following transformation. The percent of cases correctly classified were used to evaluate the performance of the classification function and the classifications were cross-validated using a jackknife procedure (leave-one-out cross-validation, SPSS Inc., Chicago, Il.). The predicted group membership of individuals based on the classification function was examined to determine into which group individuals were misclassified.

Figure 5.--Plot of the discriminant scores for the first and second discriminant functions for each individual, as well as the group centroids, from discriminant analysis performed on age groups of monk seals across all Hawaiian Islands. The first two functions correctly classified 76.0% of the original grouped cases (P < 0.001).

Although some overlap was apparent, DFA revealed spatial separation of major age classes in blubber FA profiles when seals were combined across all atolls and islands, (Fig. 5). This analysis separated age groups with 76.0% of original grouped cases, and 72.5% of cross-validated grouped cases, correctly classified (Wilk's l < 0.001). When the subadult group was removed, adults and juveniles were separated with 90.0% success. These results indicate that especially adults and juveniles, but to some extent subadults, consumed different diets. Nevertheless, there was a large degree of individual variability and certainly overlap among groups, with the greatest differences displayed between juveniles and adults.

When age groups were divided into sexes, again across all Hawaiian Islands, there was further indication of differences among demographic groups, but with both high individual variability

and overlap (Fig. 6). This analysis separated age groups and sex with only 53.6% of original grouped cases, and 42.1% of cross-validated grouped cases, correctly classified (Wilk's l < 0.001).
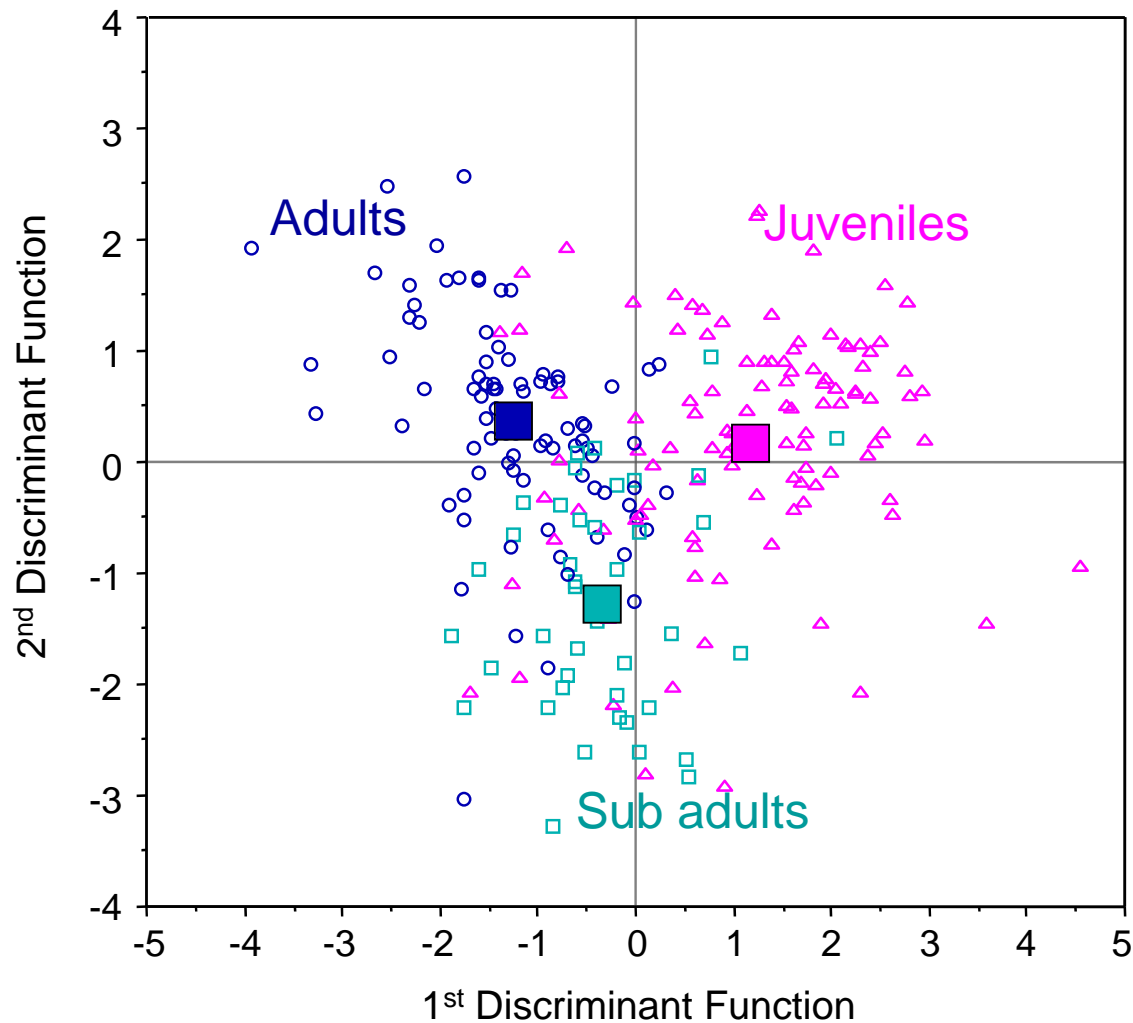

Figure 6.--Plot of the discriminant scores for the first and second discriminant functions for each individual, as well as the group centroids, from discriminant analysis performed on age groups and sex of monk seals across all Hawaiian Islands. The first two functions correctly classified 53.6% of the original grouped cases (P < 0.001).

There was little indication that juvenile and subadult males and females fed very differently, but evidence suggested only slightly greater separation in feeding habits between adult males and females.

Finally, we were limited in our ability to test differences in demographic groups across atolls and islands, given small and varying sample sizes and group representation across all areas (Table 1). However, as we had no *a priori* reason to expect that closely neighboring atolls and islands should differ considerably in their habitats and prey assemblages, we grouped seals into four general regions of the Hawaiian Islands and eliminated separation by demographic groups (Fig. 7). The four regions consisted of the western NWHI (W. NWHI), mid NWHI, eastern NWHI (E. NWHI) and the MHI.
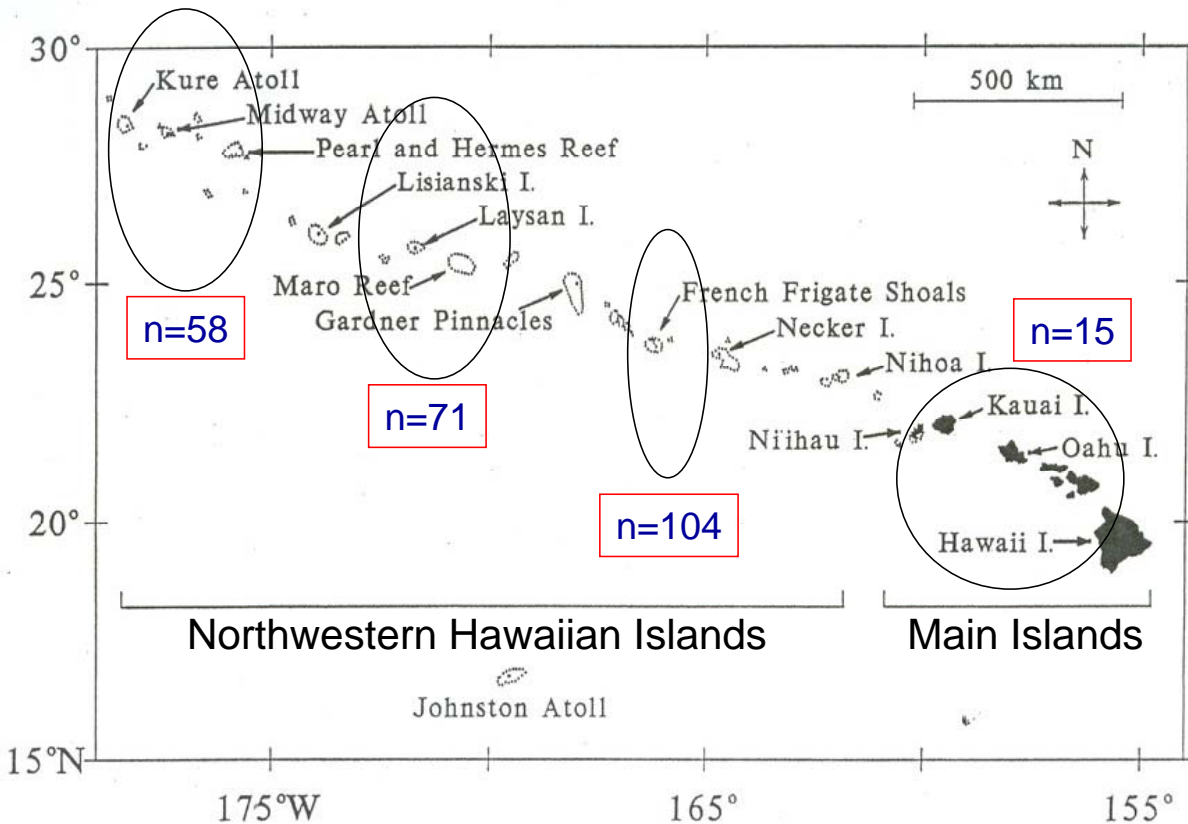
Figure 7.--Grouping of monk seals by regions within the Hawaiian Islands: W. NWHI (*n* = 58), mid NWHI (*n* = 71), E. NWHI (*n* = 104), and MHI (*n* = 15). See Table 1 for breakdown of sampling at atolls and islands and among demograhic groups.

DFA separated seals by major regions with 59.0% of original grouped cases, and 46.3% of cross-validated grouped cases, correctly classified (Wilk's l < 0.001, Fig. 8). Results suggest gradual differences occurred in diets of monk seals across the NWHI and MHI from east to west, which could be consistent with known differences in the composition of prey-species assemblages between the NWHI and MHI. Adults and juveniles, within each region, were also consistently separated, but with substantial variability. The high overlap and variability within the NWHI, suggests perhaps only slight differences in prey assemblages or feeding areas.
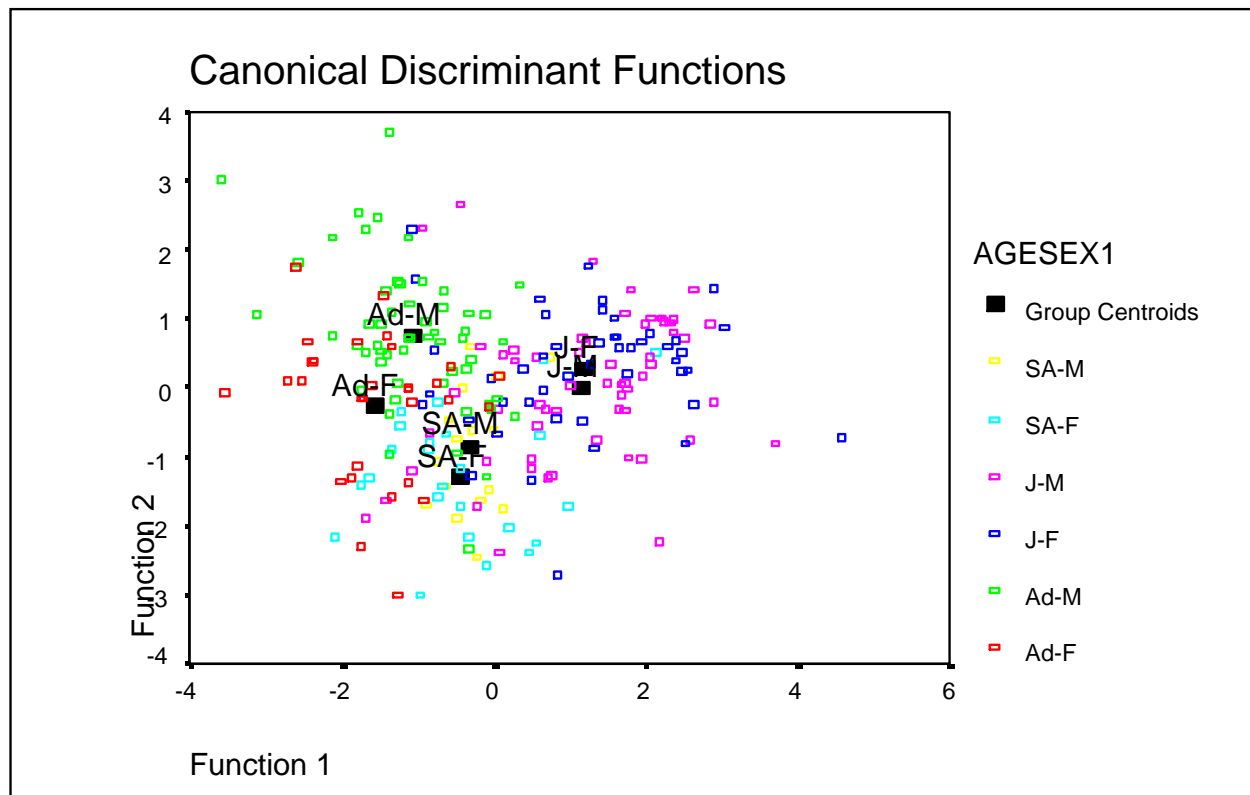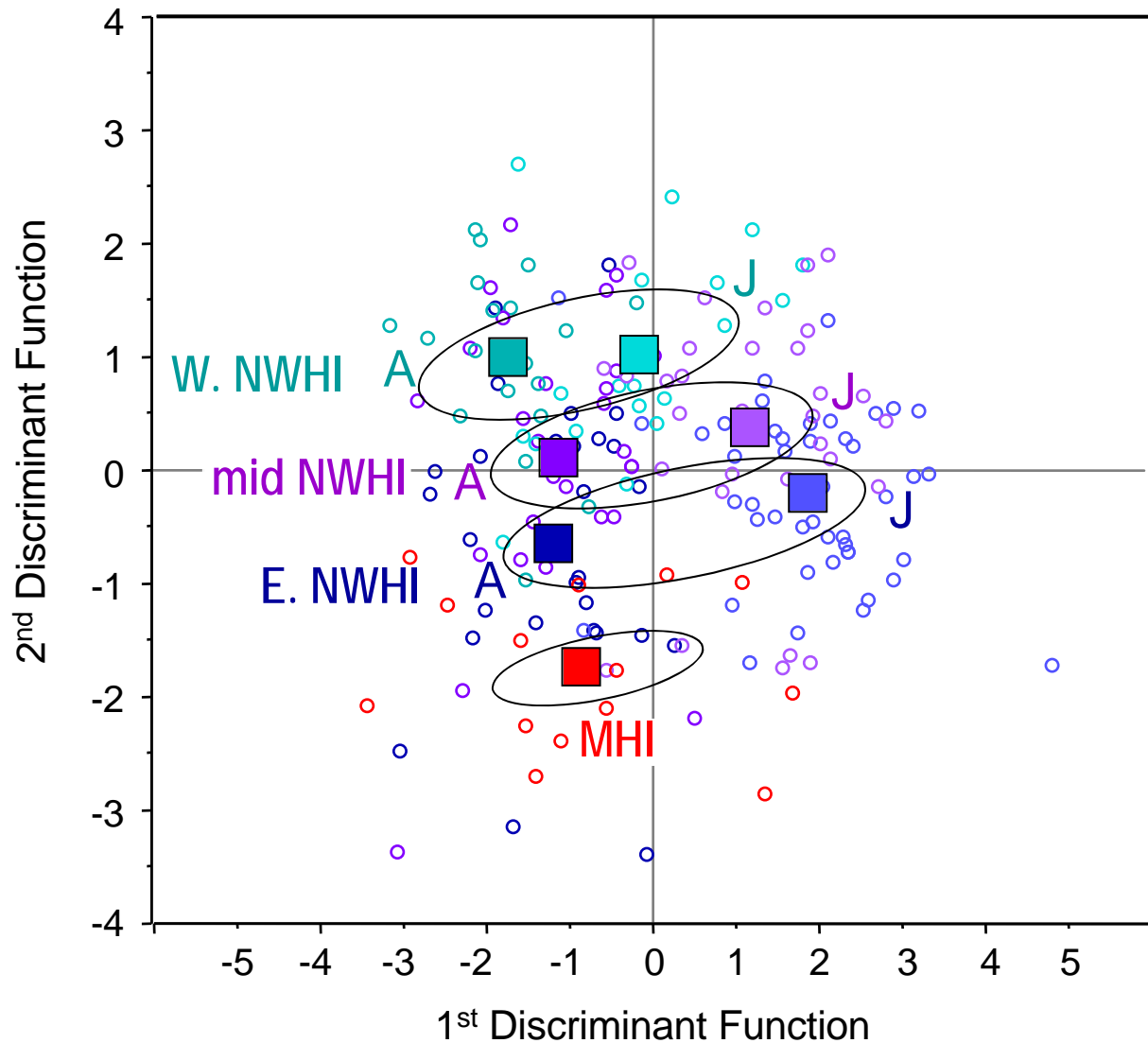
Figure 8.--Plot of the discriminant scores for the first and second discriminant functions for each individual, as well as the group centroids, from discriminant analysis performed on major regions (see Fig. 7) of monk seals in the Hawaiian Islands. The first two functions correctly classified 59.0% of the original grouped cases (P < 0.001).

In summary, although large variability was apparent among individual monk seals, we detected differences in diets between juveniles and adults, with differences between sexes being less pronounced. Differences in diets among major regions are consistent with known differences in prey-species assemblages between the MHI and NWHI. Quantitative modeling of monk seal diets using QFASA (Iverson et al., 2004), is the next step in shedding light on these qualitative variations and differences found among monk seal diets.

III.   THE PREY DATABASE FOR THE NWHI[.1]

A critical issue in the use of QFASA is the appropriate sampling and analysis of potential prey species and an understanding of the degree to which prey species can be reliably differentiated by their FA signatures in a given ecosystem. To date, all studies using QFASA have been conducted on predators in northern temperate to Arctic ecosystems. While sampling every prey species in the ecosystem is neither practical nor warranted, the onus is on the investigator to reasonably sample the range of species that is potentially important to the predator. Thus, in order to reasonably characterize the potential prey of such predators, this has required the building and analysis of prey databases in northern ecosystems ranging from fairly complex (e.g., containing up to 20-30 prey species for some seals and seabirds) to very simple (e.g., 4-8 prey species for polar bears). However, the use of QFASA in the NWHI ecosystem occupied by monk seals is greatly complicated by the sheer number of potential prey species found in this tropical environment: closer to 200 fish and invertebrate species could be potential prey, many of which share similar habitat and feeding ecology with one another, which will thus result in similar FA signatures among some species. Thus, dealing with this extreme species complexity has been the focus of the most recent work, given that it remains now the central issue in estimating monk seal diets using QFASA and in interpreting results of the model output. The key problems with such a huge number of species are that 1) not every potential prey species will be consumed or important in the diet of monk seals and therefore some sort of filter must be used to decide which will be included, 2) one could not expect that every single species could be differentiated from one another based on their FA signatures, and 3) numerical problems arise because one would be statistically modeling on more species than there are variables (FAs). Thus finding appropriate and meaningful ways to group species into appropriate and ecologically meaningful "supertaxa" is of critical importance. In the following sections of this report, we first evaluate prey FA signatures in relation to the ecology of reef to subphotic fishes and invertebrates. We then evaluate how well such groups can be reliably differentiated in the QFASA model. We use these results to model and interpret monk seal diets.

The Prey Data and Grouping Procedures

The NWHI and MHI prey database contains fat content and FA data for a total of 3130 individuals from 186 species (*see* Appendix A for species collections and Appendix B for species fat content). Prey were sampled across the full range of the NWHI, as well as the MHI, and for the time being were not separated by region, as many samples would not permit this and where sampling did permit, it would create even more "species" in QFASA modeling. Additionally, since monk seals were also sampled across all these regions, we chose to use prey from all areas.

[1]Footnote: Further work has been conducted to refine the analyses in Section III and prepare for publication in the primary literature. An update of the manuscript representing Section III and these analyses is presented in Piché et al., 2010.

The appropriate criteria for creating supertaxa is extremely important, in part due to the fact that in some cases we wish to understand the contribution of particular prey species to diets and by grouping them with certain others we may mask their contribution. Prey species FA signatures will be a function of their general ecology and dietary habits. Thus, grouping merely by closely related taxa may be meaningless. For instance, if we were to simply group all snapper species by that taxa (i.e., as "snappers"), we would be grouping both reef feeders with deep subphotic feeders and benthic carnivores with omnivores, creating a fairly different FA signature than found in any individual species. Thus, we abandoned this method.

Another means by which to group species would be to simply group those that had the most similar signatures within each group. This procedure was explored previously (Iverson, 2002). Briefly, we used hierarchical cluster analysis to investigate the similarity and differences of various species. The Kulback-Liebler (KL) distance measure was then used to determine how close two species were with respect to their FA signature. The KL distance is defined as:

$$KL = \sum (y_1 - y_2) \log(y_1/y_2)$$

where y represents each of the FAs used in the comparison, and subscripts 1 and 2 represent the two species being compared. The average linkage-method was used when performing cluster analysis, which tends to identify spherical clusters. This method produces dendograms, which indicate how similar species may be to one another and whether there are natural groups of species (i.e., groups of crustaceans, groups of butterflyfish or goatfish, etc.). Cluster analysis suffers from being fairly simplistic and based only on a single mean for each prey, but has the advantage of being able to be used with any sample size, even with an $n = 1$, allowing all species sampled to be used. These dendograms were then used to determine prey groupings using various "similarity" cut-off values using various KL distances. The drawback of this method of grouping was that while some single-family groups were produced, many of the prey groups were composed of considerably mixed species such that the resolution of diet composition using these prey groupings would be unacceptably low for evaluation of ecological significance.

From the results of previous efforts, we concluded upon a method of grouping species using somewhat of a combination of previous methods, but with emphasis on grouping taxa in the context of ecological relationships. The following procedure was followed to create the database utilized in the current series of analyses. Given the numbers of FAs that we chose to use in QFASA modeling (~ 40, see Iverson et al., 2004), we aimed to reduce the 186 species by selecting the 100 potentially most relevant species to monk seal diets and classifying these species into 40 groups based on taxonomical relatedness, diet and ecology.

The general ecological and diet guild of each species was categorized as one of five types: 1) benthic herbivores (browser, grazers), 2) planktivores (algal planktivores, zooplanktivores, gelatinous plankton), 3) benthic invertebrate feeders (corallivore, sessile invertebrates such as sponge, mobile invertebrates), 4) piscivores, or 5) detritivores/omnivores, based on data available from the literature (Parrish et al., 1986; Norris and Parrish, 1988; Parrish, 1989; Randall, 1996; Friedlander and Parrish, 1998; Hoover, 1998; FishBase). Species were then assigned to one of 40 top groups (Table 2), based upon further ecological and diet data, and grouped from a biological and ecological standpoint and where possible with species from the same family/genus (F. Parrish and R. Dollar, pers. comm.).

Table 2.--Species groupings and broad diet guild classification based on the literature for the pre-determined top 40 species groupings.

Group Name	Species	Top 40	Diet guild
Angel/cardinalfish	*Centropyge potteri* (Potter's angelfish) n = 20	1	Benthic Herbivore
	Apogon maculiferus (spotted cardinalfish) n = 18	1	Planktivore
Armorhead	*Pseudopentaceros wheeleri* (armorhead) n = 20	2	Planktivore
Beard fish	*Polymixia berndti* (Berndt's beard fish) n = 20	3	Piscivore
Bigeye	*Priacanthus alalaua* (Forskal's bigeye fish) n = 20	4	Piscivore
	Priacanthus meeki (Hawaiian bigeye) n = 20	4	Piscivore
Cusk/conger eel	*Ophidion muraenolepis* (black edged cusk eel) n = 20	5	Piscivore
	Conger cinereus (moustache conger) n = 20	5	Piscivore
	Ariosoma marginatum (big-eye conger) n = 26	5	Piscivore
Butterfly/forcep/pennantfish	*Chaetodon fremblii* (bluestripe butterflyfish) n = 22	6	Benthic Carnivore
	Chaetodon miliaris (milletseed butterflyfish) n = 27	6	Planktivore
	Chaetodon multicinctus (multiband butterflyfish) n = 19	6	Benthic Carnivore
	Chaetodon ornatissimus (ornate butterflyfish) n = 20	6	Benthic Carnivore
	Chaetodon quadrimaculatus (four spot butterflyfish) n = 20	6	Benthic Carnivore
	Forcipiger flavissimus (forcepfish) n = 26	6	Benthic Carnivore
	Heniochus diphreutes (pennantfish) n = 36	6	Planktivore

Cutthroat/snake eel	*Meadia abyssalis* (Abyssal cut-throat eel) n = 19	7	Piscivore
	Ophichthus kunaloa (snake eel) n = 20	7	Piscivore
Sergeant	*Abudefduf abdominalis* (Hawaiian sergeant) n = 20	8	Planktivore
	Abudefduf sordidus (blackspot sergeant) n = 21	8	Planktivore
	Abudefduf vaigiensis (Indo-Pacific sergeant) n = 16	8	Planktivore
Chromis/dascyllus	*Chromis ovalis* (oval chromis) n = 21	9	Planktivore
	Dascyllus albisella (Hawaiian dascyllus) n = 20	9	Planktivore
Dragonet/gurnad	*Callionymus decoratus* (longtail dragonet) n = 20	10	Benthic Carnivore
	Dactyloptena orientalis (helmut gurnard) n = 24	10	Benthic Carnivore
Goatfish (M)	*Mulloidichthys flavolineatus* (yellowstripe goatfish) n = 21	11	Benthic Carnivore
	Mulloidichthys vanicolensis (yellowfin goatfish) n = 20	11	Benthic Carnivore
Goatfish (P)	*Parupeneus bifasciatus* (doublebar goatfish) n = 19	12	Benthic Carnivore
	Parupeneus chrysonemus (yellowbarbel goatfish) n = 19	12	Benthic Carnivore
	Parupeneus multifasciatus (manybar goatfish) n = 20	12	Benthic Carnivore
	Parupeneus pleurostigma (sidespot goatfish) n = 19	12	Benthic Carnivore
Flounder	*Bothus mancus* (flowery flounder) n = 16	13	Benthic Carnivore
	Bothus pantherinus (panther flounder) n = 76	13	Benthic Carnivore

	Bothus thompsoni (Thompson's flounder) n = 25	13	Benthic Carnivore
Lizard/snakefish, sandperch	*Synodus lobeli* (Lobel's lizardfish) n = 20	14	Piscivore
	Synodus variegatus (reef lizardfish) n = 19	14	Piscivore
	Trachinocephalus myops (snakefish) n = 20	14	Piscivore
	Parapercis schauinslandii (redspotted sandperch) n = 18	14	Benthic Carnivore
Moray eel	*Gymnothorax albimarginatus* (whitemargin moray) n = 20	15	Piscivore
	Gymnothorax berndti (Berndt's moray) n = 20	15	Piscivore
	Gymnothorax eurostus (stout moray) n = 14	15	Piscivore
	Gymnothorax flavimarginatus (yellowmargin moray) n = 19	15	Piscivore
	Gymnothorax meleagris (whitemouth moray) n = 18	15	Piscivore
	Gymnothorax steindachneri (Steindachner's moray) n = 25	15	Piscivore
	Gymnothorax undulatus (undulated moray) n = 19	15	Piscivore
Parrotfish	*Chlorurus perspicillatus* (spectacled parrotfish) n = 22	16	Benthic Herbivore
	Chlorurus sordidus (bullethead parrotfish) n = 19	16	Benthic Herbivore
	Scarus dubius (regal parrotfish) n = 19	16	Benthic Herbivore
Toby	*Canthigaster coronata* (crown toby) n = 20	17	Benthic Carnivore
	Canthigaster jactator (Hawaiian whitespotted toby) n = 24	17	Benthic Carnivore

	Canthigaster rivulata (maze toby) n = 16	17	Benthic Carnivore
	Torquigner florealis (floral puffer) n = 20	17	Benthic Carnivore
Chub	*Kyphosus bigibbus* (gray chub) n = 19	18	Benthic Herbivore
	Kyphosus vaigiensis (lowfin chub) n = 19	18	Benthic Herbivore
Scorpionfish	*Sebastapistes ballieui* (spotfin scorpionfish) n = 19	19	Benthic Carnivore
Squirrelfish snapper	*Etelis carbunculus* (squirrelfish snapper) n = 21	20	Benthic Carnivore *(deep)*
Bluestripe snapper	*Lutjanus kasmira* (bluestripe snapper) n = 22	21	Omnivore *(shallow, invasive)*
Pink snapper	*Pristipomoides filamentosus* (pink snapper) n = 24	22	Benthic Carnivore *(important fishery)*
Flower snapper	*Pristipomoides zonatus* (flower snapper) n = 19	23	Omnivore *(deep)*
Soldierfish	*Myripristis berndti* (bigscale soldierfish) n = 20	24	Benthic Carnivore
Squirrelfish	*Sargocentron xantherythrum* (Hawaiian squirrelfish) n = 21	25	Benthic Carnivore
	Acanthurus achilles (achilles tang) n = 20	26	Benthic Herbivore
	Acanthurus dussumieri (eyestripe surgeonfish) n = 16	26	Benthic Herbivore
	Acanthurus leucopareius (whitebar surgeonfish) n = 22	26	Benthic Herbivore
	Acanthurus nigroris (bluelined surgeonfish) n = 20	26	Benthic Herbivore
	Acanthurus olivaceus (orangeband surgeonfish) n = 20	26	Benthic Herbivore
Tang/surgeonfish	*Acanthurus triostegus* (convict tang) n = 20	26	Benthic Herbivore

	Ctenochaetus strigosus (goldring surgeonfish) n = 16	26	Benthic Herbivore
	Zebrasoma flavescens (yellow tang) n = 20	26	Benthic Herbivore
Unicornfish	*Naso lituratus* (orangespine unicornfish) n = 19	27	Benthic Herbivore
	Naso unicornis (bluespine unicornfish) n = 19	27	Benthic Herbivore
Tile/knife/razorfish	*Malacanthus brevirostris* (flagtail tilefish) n = 16	28	Benthic Carnivore
	Cymolutes lecluse (Hawaiian knifefish) n = 23	28	Benthic Carnivore
	Inistius pavo (peacock razorfish) n = 15	28	Benthic Carnivore
	Inistius umbrilatus (blackside razorfish) n = 19	28	Benthic Carnivore
Triggerfish	*Melichthys niger* (black triggerfish) n = 20	29	Planktivore
	Sufflamen bursa (lei triggerfish) n = 19	29	Benthic Carnivore
Wrasse/hogfish/coris	*Anampses cuvier* (pearl wrasse) n = 20	30	Benthic Carnivore
	Bodianus bilunulatus (Hawaiian hogfish) n = 20	30	Benthic Carnivore
	Coris ballieui (lined coris) n = 16	30	Benthic Carnivore
	Coris flavovittata (yellowstriped coris) n = 20	30	Benthic Carnivore
	Oxycheilinus unifasciatus (ringtail wrasse) n = 19	30	Benthic Carnivore
	Thalassoma ballieui (blacktail wrasse) n = 20	30	Benthic Carnivore
	Thalassoma dupery (saddle wrasse) n = 20	30	Benthic Carnivore
Octopus	*Octopus cyanea* (Hawaiian day octopus) n = 13	31	Benthic Carnivore
	Octopus ornatus (ornate octopus) n = 4	31	Benthic Carnivore
	Octopus sp. n = 23	31	Benthic Carnivore

Squid	*Sthenoteuthis oualaniensis* (neon flying squid) n = 15	32	Piscivore
Shrimp	*Heterocarpus ensifer* (two-spined shrimp) n = 31	33	Benthic Carnivore
	Heterocarpus laevigatus (red-tipped shrimp) n = 39	33	Benthic Carnivore
Box crab	*Calappa bicornis* (two-horned box crab) n = 23	34	Benthic Carnivore
	Calappa calappa (smooth box crab) n = 36	34	Benthic Carnivore
Swimming crab	*Charybdis hawaiiensis* (Hawaiian swimming crab) n = 29	35	Benthic Carnivore
Pebble crab	*Carpilius convexus* (convex pebble crab) n = 21	36	Benthic Carnivore
Slipper lobster	*Scyllarides haanii* (ridgeback slipper lobster) n = 30	37	Benthic Carnivore
	Scyllarides squammosus (common slipper lobster) n = 72	37	Benthic Carnivore
Spiny lobster	*Panulirus marginatus* (spiny lobster) n = 60	38	Benthic Carnivore
Boarfish	*Antigonia eos* (boar fish) n = 10	39	Benthic Carnivore
	Antigonia capros (boar fish) n = 10	39	Benthic Carnivore
Duckbill	*Bembrops filifera* (duck-billed bembropsid) n = 20	40	Benthic Carnivore

Descriptive Statistics of Prey Groupings and Discriminant Analyses

Of the 40 prey groups located in the various ecological subsystems encountered throughout the NWHI range, most reside in reef (47.4%) or bank (39.8%) areas, with 2.9% and 8.5% occupying slope and subphotic areas, respectively. Of the four typical habitats encountered, 49.0% of species groups occupy reef habitat, with 26.1%, 11.4% and 12.8% found in sand, rock and carbonate habitats, respectively. Of the diet guilds, 56.5% of species groups were classified as benthic carnivores, 17.6% as piscivores, 14.1% as benthic herbivores, 9.9% as planktivores and 1.9% as detrivores.

The species groupings with the smallest sample size (the squid *Sthenoteuthis oualaniensis, n =* 15) dictated that only 14 FAs (with 18:0 which was used for establishing the log ratios) could be used for the discriminant analyses (n-1 of the smallest sample size). Fourteen FAs were, therefore, selected for the analysis on the basis of their origin (entirely or mostly dietary as apposed to biosynthesized), and greatest abundance and variance (14:0, 16:0, 16:1n-7, 18:1n-9, 18:1n-7, 18:2n-6, 20:1n-9, 20:4n-6, 20:5n-3, 22:1n-11, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3). This subsample of FAs was subsequently re-normalized over 100% (each FA was divided by the sum of all FAs for a given signature). The re-normalized values were then divided by 18:0 (as reference) to obtain ratios. A value of 0.005 was added to any zero values to allow further analyses. The log of each ratio was taken, and the resulting "log ratios" were used in the DFA using SPSS (see Section II for further description of DFA and log ratios).

The initial analysis using all 40 species groups illustrates the spatial distribution of each group relative to one another (Fig. 9). Herbivores, crustaceans, and benthic carnivores and piscivores formed broadly clustered groupings. Cluster 1 encompassed the herbivores *Acanthurus* spp (surgeonfish), *Naso* spp. (unicornfish), *Chlorurus* and *Scarus* (parrotfish) and *Kyphosus* (chubs), but also included the *Octopus* spp. Cluster 2 encompassed all crustaceans *Heterocarpus* spp (shrimps), *Calappa* spp. (box crabs), *Charybdis* spp. (swimming crabs), *Carpillius* spp. (xanthid crabs), *Scyllarides* spp. (slipper lobsters) and *Panulirus marginatus* (spiny lobsters). Cluster 3 encompassed the remaining benthic carnivores and piscivores, excluding *Pseudopentaceros wheeleri* (slender armorhead), which clustered by itself. Table 3 identifies the classification success for each species and underlines the more problematic species with higher proportions of misclassified individuals. Despite such broad overlap, a number of species were reasonably well classified at > 75% success. In a number of cases, misclassified individuals were classified as closely related species (i.e., crustaceans for other crustaceans, eels for other eels).
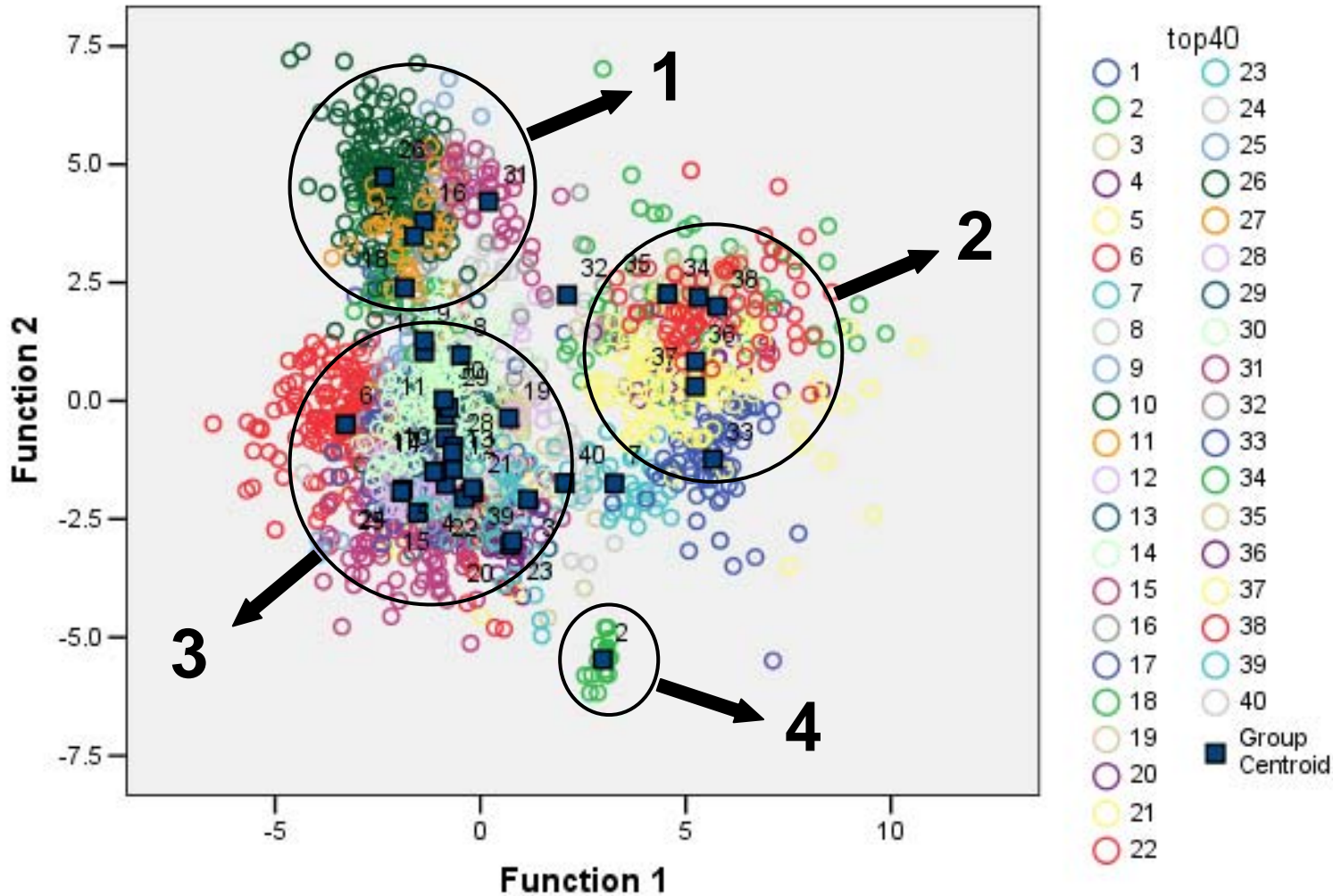
Figure 9.--Scatter plot of the discriminant analysis on the top 40 species groups (see Table 2), yielded a cross-validated classification success of 72%. 1= Herbivore cluster (but includes the carnivorous Octopuses), 2 = Crustacean cluster, 3 = Benthic carnivores and piscivores cluster; 4 = *Pseudopentaceros wheeleri* (slender armorhead) was the only species with 100% classification success that was spatially isolated from all other species.

Table 3.--Results of discriminant analysis classification for each species group (*see* Table 2, Fig. 9). Includes the top 40 groupings, diet guild, individual count, proportion of individuals correctly classified (%), and major misclassification for each species. Highlighted in yellow are the groups where the predicted group membership was less than 50% accurate.

Species	Top 40	Diet guild	Count	Proportion Correctly classified	Major misclassification
Centropyge potteri (Potter's angelfish)	1	Benthic Herbivore	21/38	55.3%	6 misclassified as group 10
Apogon maculiferus (spotted cardinalfish)	1	Planktivores			
Pseudopentaceros wheeleri (armorhead)	2	Planktivores	20/20	100%	------------------
Polymixia berndti (Berndt's beard fish)	3	Piscivores	16/20	80%	
Priacanthus alalaua (Forskal's bigeye fish)	4	Piscivores	28/40	70%	4 misclassified as group 12
Priacanthus meeki (Hawaiian bigeye)	4	Piscivores			
Ophidion muraenolepis (black edged cusk eel)	5	Piscivores	24/66	36.4%	9 misclassified as a 14 14 misclassified as group 15 (other eels) 8 misclassified as group 28
Conger cinereus (moustache conger)	5	Piscivores			
Ariosoma marginatum (big-eye conger)	5	Piscivores			
Chaetodon fremblii (bluestripe butterflyfish)	6	Benthic Carnivores	104/170	61.2%	8 misclassified as group 5 9 misclassified as group 15 10 misclassified as group 24 8 misclassified as group30 12 misclassified as group 39
Chaetodon miliaris (milletseed butterflyfish)	6	Planktivores			
Chaetodon multicinctus (multiband butterflyfish)	6	Benthic Carnivores			
Chaetodon ornatissimus (ornate butterflyfish)	6	Benthic Carnivores			
Chaetodon quadrimaculatus (four spot butterflyfish)	6	Benthic Carnivores			
Forcipiger flavissimus (forcepfish)	6	Benthic Carnivores			
Heniochus diphreutes (pennantfish)	6	Planktivores			

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Meadia abyssalis (Abyssal cut-throat eel)	7	Piscivores	37/39	94.9%	
Ophichthus kunaloa (snake eel)	7	Piscivores			
Abudefduf abdominalis (Hawaiian sergeant)	8	Planktivores	29/57	50.9%	12 misclassified as group 9 5 misclassified as group 13
Abudefduf sordidus (blackspot sergeant)	8	Planktivores			
Abudefduf vaigiensis (Indo-Pacific sergeant)	8	Planktivores			
Chromis ovalis (oval chromis)	9	Planktivores	18/41	<mark>43.9%</mark>	5 misclassified as group 8 4 misclassifed as group 10 7 misclassified as group 26
Dascyllus albisella (Hawaiian dascyllus)	9	Planktivores			
Callionymus decoratus (longtail dragonet)	10	Benthic Carnivores	30/44	68.2%	4 misclassified as group 11 4 misclassified as group 28
Dactyloptena orientalis (helmut gurnard)	10	Benthic Carnivores			
Mulloidichthys flavolineatus (yellowstripe goatfish)	11	Benthic Carnivores	30/41	73.2%	3 misclassified as group 12 2 misclassified as group 14
Mulloidichthys vanicolensis (yellowfin goatfish)	11	Benthic Carnivores			
Parupeneus bifasciatus (doublebar goatfish)	12	Benthic Carnivores	37/77	<mark>48.1%</mark>	8 misclassified as group 11 5 misclassified as group 19 5 misclassified as group 24
Parupeneus chrysonemus (yellowbarbel goatfish)	12	Benthic Carnivores			
Parupeneus multifasciatus (manybar goatfish)	12	Benthic Carnivores			
Parupeneus pleurostigma (sidespot goatfish)	12	Benthic Carnivores			

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Bothus mancus (flowery flounder)	13	Benthic Carnivores	84/116	72.4%	8 misclassified as group 5 5 misclassified as group 17 8 misclassified as group 22
Bothus pantherinus (panther flounder)	13	Benthic Carnivores			
Bothus thompsoni (Thompson's flounder)	13	Benthic Carnivores			
Synodus lobeli (Lobel's lizardfish)	14	Piscivores	53/77	68.8%	7 misclassified as group 10 8 misclassified as group 21
Synodus variegatus (reef lizardfish)	14	Piscivores			
Trachinocephalus myops (snakefish)	14	Piscivores			
Parapercis schauinslandii (redspotted sandperch)	14	Benthic carnivores			
Gymnothorax albimarginatus (whitemargin moray)	15	Piscivores	113/149	75.8%	5 misclassified as group 5 5 misclassified as group 17
Gymnothorax berndti (Berndt's moray)	15	Piscivores			
Gymnothorax eurostus (stout moray)	15	Piscivores			
Gymnothorax flavimarginatus (yellowmargin moray)	15	Piscivores			
Gymnothorax meleagris (whitemouth moray)	15	Piscivores			
Gymnothorax steindachneri (Steindachner's moray)	15	Piscivores			
Gymnothorax undulatus (undulated moray)	15	Piscivores			
Chlorurus perspicillatus (spectacled parrotfish)	16	Benthic Herbivores	55/60	91.7%	4 misclassified as group 1
Chlorurus sordidus (bullethead parrotfish)	16	Benthic Herbivores			

Scarus dubius (regal parrotfish)	16	Benthic Herbivores			
Canthigaster coronata (crown toby)	17	Benthic Carnivores			
Canthigaster jactator (Hawaiian whitespotted toby)	17	Benthic Carnivores	62/80	77.5%	7 misclassified as group 29
Canthigaster rivulata (maze toby)	17	Benthic Carnivores			
Torquigner florealis (floral puffer)	17	Benthic Carnivores			
Kyphosus bigibbus (gray chub)	18	Benthic Herbivores	33/38	86.8%	
Kyphosus vaigiensis (lowfin chub)	18	Benthic Herbivores			
Sebastapistes ballieui (spotfin scorpionfish)	19	Benthic Carnivores	19/19	100%	_____
Etelis carbunculus (squirrelfish snapper)	20	Benthic Carnivores	19/21	90.5%	
Lutjanus kasmira (bluestripe snapper)	21	Omnivores	15/22	68.2%	
Pristipomoides filamentosus (pink snapper)	22	Benthic Carnivores	16/24	66.7%	6 misclassified as group 23
Pristipomoides zonatus (flower snapper)	23	Omnivores	18/19	94.7%	
Myripristis berndti (bigscale soldierfish)	24	Benthic Carnivores	16/20	80%	
Sargocentron xantherythrum (Hawaiian squirrelfish)	25	Benthic Carnivores	14/21	66.7%	4 misclassified as group 24
Acanthurus achilles (achilles tang)	26	Benthic Herbivores			
Acanthurus dussumieri (eyestripe surgeonfish)	26	Benthic Herbivores			
Acanthurus leucopareius (whitebar surgeonfish)	26	Benthic Herbivores	140/154	90.9%	7 misclassified as group 9 5 misclassified as 1
Acanthurus nigroris (bluelined surgeonfish	26	Benthic Herbivores			

Species	Group	Diet	Ratio	Percent	Misclassifications
Acanthurus olivaceus (orangeband surgeonfish)	26	Benthic Herbivores			
Acanthurus triostegus (convict tang)	26	Benthic Herbivores			
Ctenochaetus strigosus (goldring surgeonfish)	26	Benthic Herbivores			
Zebrasoma flavescens (yellow tang)	26	Benthic Herbivores			
Naso lituratus (orangespine unicornfish)	27	Benthic Herbivores			
Naso unicornis (bluespine unicornfish)	27	Benthic Herbivores	36/38	94.7%	
Malacanthus brevirostris (flagtail tilefish)	28	Benthic Carnivores			
Cymolutes lecluse (Hawaiian knifefish)	28	Benthic Carnivores	31/73	<mark>42.5%</mark>	6 misclassified as group 11 8 misclassified as group 12 6 misclassified as group 17 5 misclassified as group 19
Inistius pavo (peacock razorfish)	28	Benthic Carnivores			
Inistius umbrilatus (blackside razorfish)	28	Benthic Carnivores			
Melichthys niger (black triggerfish)	29	Planktivores			
Sufflamen bursa (lei triggerfish)	29	Benthic Carnivores	35/39	89.7%	
Anampses cuvier (pearl wrasse)	30	Benthic Carnivores			
Bodianus bilunulatus (Hawaiian hogfish)	30	Benthic Carnivores			5 misclassified as group 5 6 misclassified as group 2810 misclassified as group 11 10 misclassified as group 19
Coris ballieui (lined coris)	30	Benthic Carnivores			
Coris flavovittata (yellowstriped coris)	30	Benthic Carnivores	88/135	65.2%	
Oxycheilinus unifasciatus (ringtail wrasse)	30	Benthic Carnivores			
Thalassoma ballieui (blacktail wrasse)	30	Benthic Carnivores			
Thalassoma duperry (saddle wrasse)	30	Benthic Carnivores			

Octopus cyanea (Hawaiian day octopus)	31	Benthic Carnivores	34/36	94.4%	2 misclassified as group 32
Octopus ornatus (ornate octopus)	31	Benthic Carnivores			
Octopus sp.	31	Benthic Carnivores			
Sthenoteuthis oualaniensis (neon flying squid)	32	Piscivores	15/15	100%	————————
Heterocarpus ensifer (two-spined shrimp)	33	Benthic Carnivores	70/70	100%	————————
Heterocarpus laevigatus (red-tipped shrimp)	33	Benthic Carnivores			
Calappa bicornis (two-horned box crab)	34	Benthic Carnivores	24/59	40.7%	19 misclassified as group 38 (spiny lobster) 6 misclassified as group 36 (xanthid crabs)
Calappa calappa (smooth box crab)	34	Benthic Carnivores			
Charybdis hawaiiensis (Hawaiian swimming crab)	35	Benthic Carnivores	24/29	82.8%	
Carpilius convexus (convex pebble crab)	36	Benthic Carnivores	16/21	76.2%	
Scyllarides haanii (ridgeback slipper lobster)	37	Benthic Carnivores	75/102	73.5%	14 misclassified as group 36
Scyllarides squammosus (common slipper lobster)	37	Benthic Carnivores			
Panulirus marginatus (spiny lobster)	38	Benthic Carnivores	46/71	64.8%	8 misclassified as group 36 13 misclassified as group 34 (Calappa spp)
Antigonia eos (boar fish)	39	Benthic Carnivores	20/20	100%	————————
Antigonia capros (boar fish)	39	Benthic Carnivores			
Bembrops filifera (duck-billed bembropsid)	40	Benthic Carnivores	16/19	84.2%	3 misclassified as group 20

Separate Analyses of Clusters

A DFA was subsequently conducted on each of the three major clusters, separately, to further evaluate the degree of within-cluster spatial distribution and the predicted classification success within each cluster.

Cluster 1: the Herbivore Cluster. Figure 10 illustrates the degree of spatial division among the different species grouping in cluster 1. Moreover, the classification success was very high, with 99% of the individuals correctly classified. The classification results for cluster 1 are reported in detail in Table 4.

Cluster 2: the Crustacean Cluster. Figure 11 illustrates the spatial division among the groups of species within cluster 2. The classification success was high, with 82% of the individuals correctly classified. The species grouping with the lowest classification success was the box crabs (*Calappa* spp) with only 57.6% of the individuals correctly classified; 23% of the box crabs were misclassified as spiny lobsters. The detailed classification results for cluster 2 are reported in Table 5.

Cluster 3: The Carnivore/Piscivore Cluster. Figure 12 illustrates the relatively poor spatial division among the groups of species within cluster 3. This cluster yielded the lowest classification success (66%), but is also the one encompassing the highest number of species/species groupings, which renders the analyses much more complex. The classification results for cluster 3 are reported in detail in Table 6.

Canonical Discriminant Functions

16 = Parrotfishes (Chlorurus, Scarus spp.)
18= Chubs (Kyphosus spp.)
26= Tangs & surgeonfishes (Acanthurus, Ctenochaetus Zebrasoma spp.)
27 = Unicornfishes (Naso spp.)
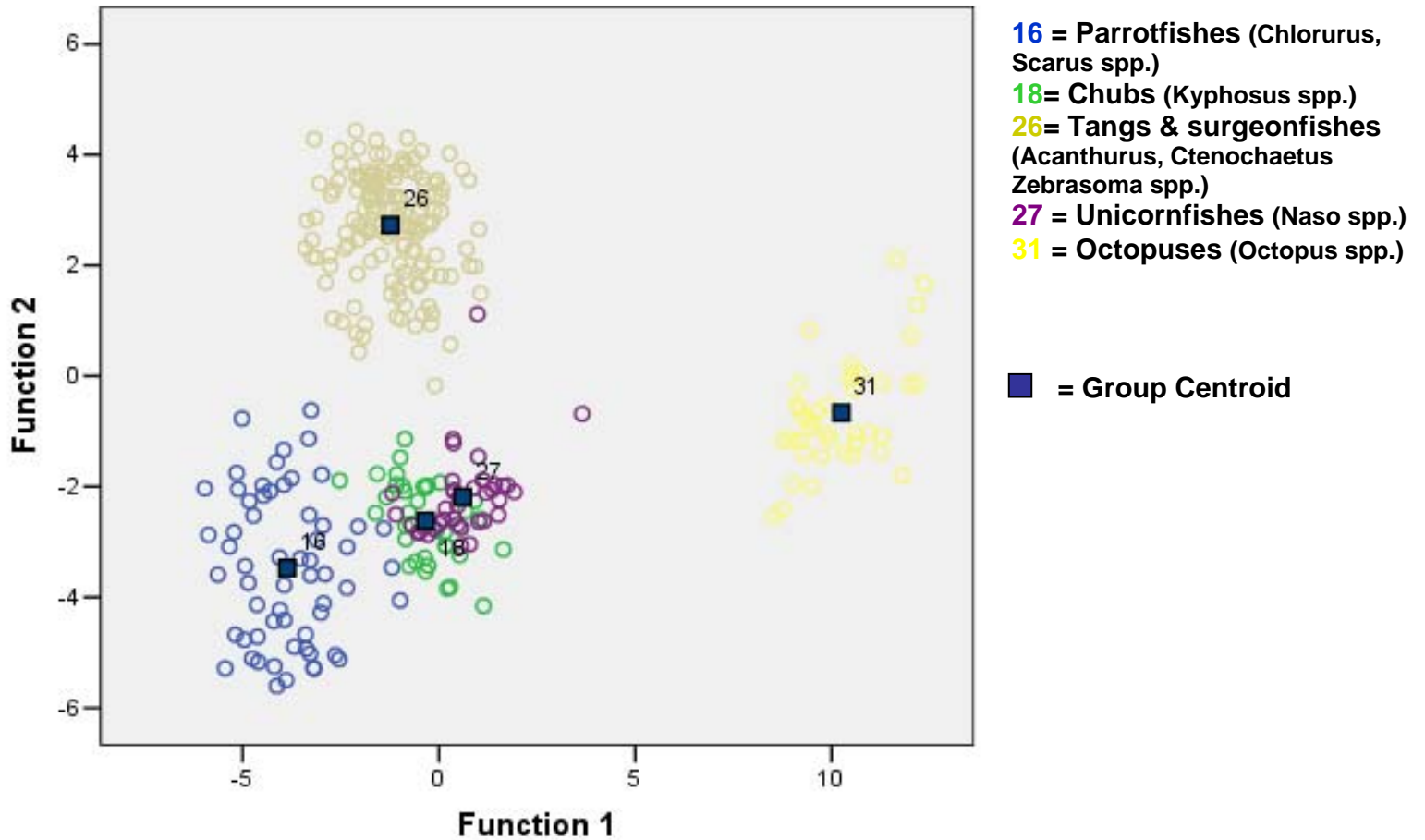31 = Octopuses (Octopus spp.)

■ = Group Centroid

Figure 10.--Scatter plot of the discriminant analysis on the herbivore cluster yielding a cross-validated classification success of 99%.

Table 4.--Discriminant analysis classification results for the herbivore cluster (Fig. 10). Includes the top 40 groupings corresponding to the groups used in the within-cluster discriminant analysis, the diet guild, the ecological subsystem, the individual count, and the proportion of individuals correctly classified (%).

Species	Top40	Diet Guild	Ecological Subsystem	Count	Proportion correctly classified (%)
Chlorurus perspicillatus (spectacled parrotfish)					
Chlorurus sordidus (bullethead parrotfish)	16	Benthic Herbivores	Reef	60/60	100%
Scarus dubius (regal parrotfish)					
Kyphosus bigibbus (gray chub)	18	Benthic Herbivores	Reef	38/38	100%
Kyphosus vaigiensis (lowfin chub)					
Acanthurus achilles (achilles tang)					
Acanthurus dussumieri (eyestripe surgeonfish)					
Acanthurus leucopareius (whitebar surgeonfish)					
Acanthurus nigroris (bluelined surgeonfish)	26	Benthic Herbivores	Reef	151/153	98.7%
Acanthurus olivaceus (orangeband surgeonfish)					
Acanthurus triostegus (convict tang)					
Ctenochaetus strigosus (goldring surgeonfish)					
Zebrasoma flavescens (yellow tang)					
Naso lituratus (orangespine unicornfish)	27	Benthic Herbivores	Reef	37/38	97.4%
Naso unicornis (bluespine unicornfish)					
Octopus cyanea (Hawaiian day octopus)	31	Benthic Carnivores	Bank	40/40	100%
Octopus ornatus (ornate octopus)					
Octopus sp.					

Figure. 11.--Scatter plot of the discriminant analysis on the crustacean cluster yielding a cross-validated classification success of 82%.
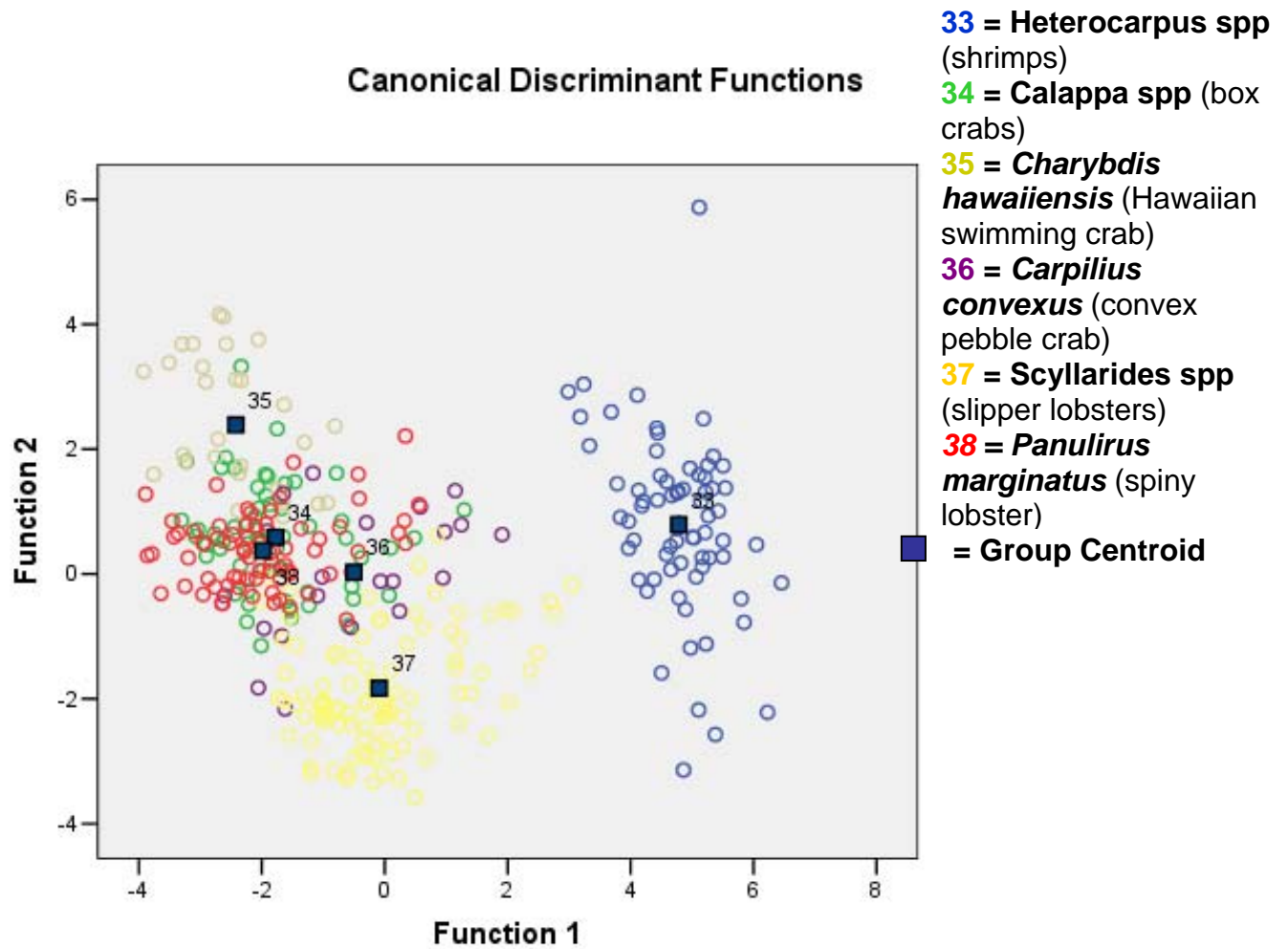
Table 5.--Discriminant analysis classification results for the crustacean cluster (Fig. 11). Includes the top 40 groupings used in the within cluster discriminant analysis, the diet guild, the ecological subsystem, the individual count, the proportion of individuals correctly classified (%), and the major misclassifications.

Species	Top40	Diet Guild	Ecological Subsystem	Count	Proportion correctly classified (%)	Major Misclassification
Heterocarpus ensifer (two-spined shrimp) *Heterocarpus laevigatus* (red-tipped shrimp)	33	Benthic Carnivores	Subphotic	70/70	100%	_____
Calappa bicornis (two-horned box crab) *Calappa calappa* (smooth box crab)	34	Benthic Carnivores	Bank	34/59	57.6%	14 misclassified as 38 (38 = spiny lobster)
Charybdis hawaiiensis (Hawaiian swimming crab)	35	Benthic Carnivores	Bank	27/29	93.1%	
Carpilius convexus (convex pebble crab)	36	Benthic Carnivores	Bank	16/21	76.2%	4 misclassified as 38
Scyllarides haanii (ridgeback slipper lobster) *Scyllarides squammosus* (common slipper lobster)	37	Benthic Carnivores	Bank	89/102	87.3%	4 misclassified as 36 4 misclassified as 38
Panulirus marginatus (spiny lobster)	38	Benthic Carnivores	Reef	51/71	71.8%	12 misclassified as 34

Canonical Discriminant Functions
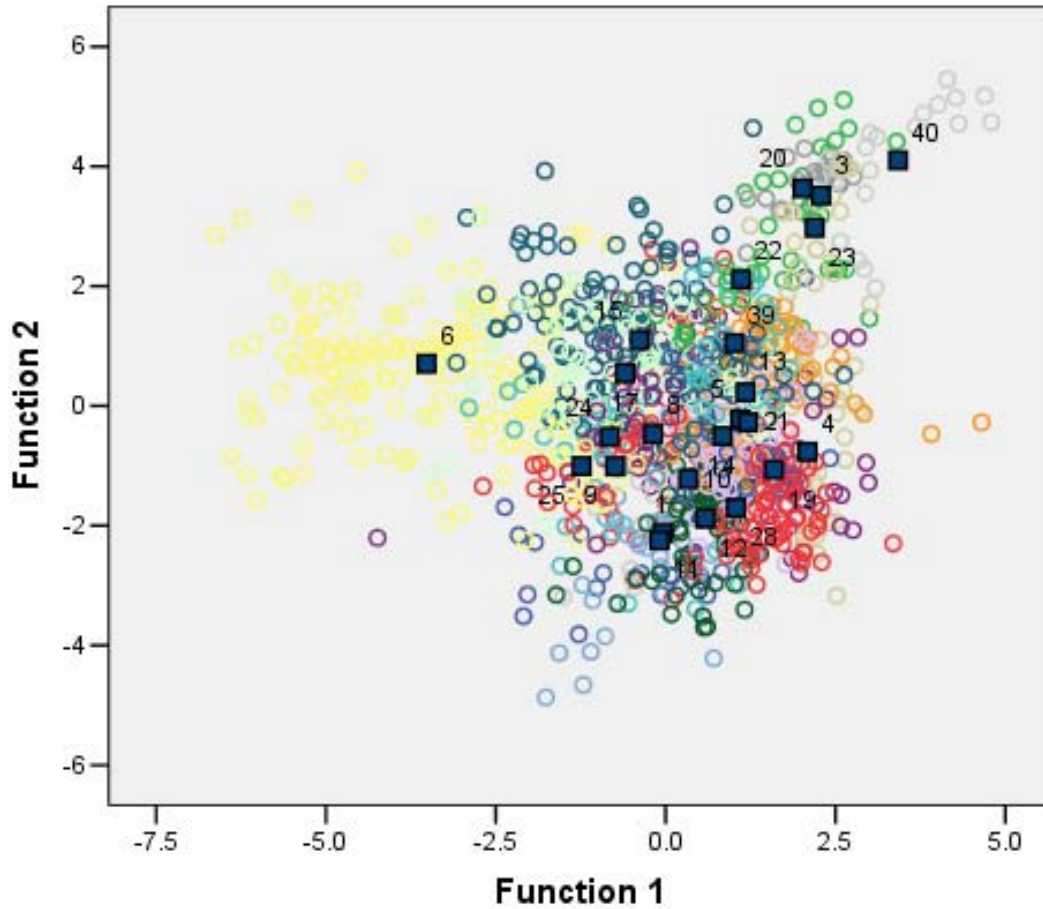
1 = Angelfishes & Cardinalfishes
3 = Beardfishes
4 = Bigeyes
5 = Cusk eels & Conger eels
6 = Butterflyfishes, Forcepfishes & Pennantfishes
8 = Sergeants
9 = Damselfishes
10 = Dragonets, Helmet gurnards
11 = Goatfishes (Mulloidichthys spp)
12 = Goatfishes (Parupeneus spp)
13 = Lefteye flounder
14 = Lizardfishes & Snakefishes
15 = Moray eels
17 = Pufferfishes
19 = Scorpionfishes
20 = Squirrelfish snappers
21 = Bluestripe snappers
22 = Pink snappers
23 = Flower snappers
24 = Bigscale soldierfishes
25 = Hawaiian squirrelfishes
28 = Tilefishes, Knifefishes, & Peacock wrasse
39 = Boarfishes
40 = Duck-billed bembropsid

Group Centroid

Figure 12.--Scatter plot of the discriminant analysis on the carnivore/piscivore cluster yielding a cross-validated classification success of 66%.

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Table 6.--Discriminant analysis classification results for the carnivore/piscivore cluster (Fig. 12). Includes the top 40 groupings used in the within cluster discriminant analysis, the diet guild, the ecological subsystem, the individual count, the proportion of individuals correctly classified (%), and the major misclassifications. Highlighted in yellow are the groups where the predicted group membership was less than 50% accurate.

Species	Top40	Diet Guild	Ecological Subsystem	Count	Proportion correctly classified (%)	Major Misclassification
Centropyge potteri (Potter's angelfish)	1	Benthic Herbivores	Bank	20/38	52.6%	6 misclassified as 28
Apogon maculiferus (spotted cardinalfish)		Planktivores	Bank			
Polymixia berndti (Berndt's beard fish)	3	Piscivores	Subphotic	18/20	90%	
Priacanthus alalaua (Forskal's bigeye fish)	4	Piscivores	Bank	31/40	77.5%	
Priacanthus meeki (Hawaiian bigeye)						
Ophidion muraenolepis (black edged cusk eel)	5	Piscivores	Bank	14/66	27.3%	7 misclassified as 14 16 misclassified as 28 15 misclassified as 15
Conger cinereus (moustache conger)						
Ariosoma marginatum (big-eye conger)						
Chaetodon fremblii (bluestripe butterflyfish)	6	Benthic Carnivores	Reef	128/170	75.3%	9 misclassified as 24 7 misclassified as 25
Chaetodon miliaris (milletseed butterflyfish)		Planktivores	Reef			
Chaetodon multicinctus (multiband butterflyfish)		Benthic Carnivores	Reef			
Chaetodon ornatissimus (ornate butterflyfish)		Benthic Carnivores	Reef			
Chaetodon quadrimaculatus (four spot butterflyfish)		Benthic Carnivores	Reef			
Forcipiger flavissimus (forcepfish)		Benthic Carnivores	Reef			
Heniochus diphreutes (pennantfish)		Planktivores	Bank			
Abudefduf abdominalis (Hawaiian sergeant)	8	Planktivores	Reef	34/57	59.6%	13 misclassified as 9
Abudefduf sordidus (blackspot sergeant)		Planktivores	Reef			
Abudefduf vaigiensis		Planktivores	N/A			

Species	Top40	Diet Guild	Ecological Subsystem	Count	Proportion correctly classified (%)	Major Misclassification
(Indo-Pacific sergeant)						
Chromis ovalis (oval chromis)	9	Planktivores	Reef			5 misclassified as 8
Dascyllus albisella (Hawaiian dascyllus)						
Callionymus decoratus (longtail dragonet)	10	Benthic Carnivores	Bank	30/44	68.2%	8 misclassified as 11
Dactyloptena orientalis (helmut gurnard)						
Mulloidichthys flavolineatus (yellowstripe goatfish)	11	Benthic Carnivores	Reef	25/41	61%	7 misclassified as 12
Mulloidichthys vanicolensis (yellowfin goatfish)						
Parupeneus bifasciatus (doublebar goatfish)						
Parupeneus chrysonemus (yellowbarbel goatfish)	12	Benthic Carnivores	Reef	39/77	50.6%	10 misclassified as 11 8 misclassified as 19
Parupeneus multifasciatus (manybar goatfish)						
Parupeneus pleurostigma (sidespot goatfish)						
Bothus mancus (flowery flounder)						
Bothus pantherinus (panther flounder)	13	Benthic Carnivores	Bank	93/116	80.2%	10 misclassified as 5
Bothus thompsoni (Thompson's flounder)						
Synodus lobeli (Lobel's lizardfish)						
Synodus variegatus (reef lizardfish)	14	Piscivores	Bank	50/77	64.9%	6 misclassified as 10
Trachinocephalus myops (snakefish)						
Parapercis schauinslandii (redspotted sandperch)						
Gymnothorax albimarginatus (whitemargin moray)						
Gymnothorax berndti (Berndt's moray)						
Gymnothorax eurostus	15	Piscivores	Reef	102/149	68.5%	8 misclassified as 17

Species	Top40	Diet Guild	Ecological Subsystem	Count	Proportion correctly classified (%)	Major Misclassification
(stout moray)						8 misclassified as 5
Gymnothorax flavimarginatus (yellowmargin moray)						
Gymnothorax meleagris (whitemouth moray)						
Gymnothorax steindachneri (Steindachner's moray)						
Gymnothorax undulatus (undulated moray)						
Canthigaster coronata (crown toby)						
Canthigaster jactator (Hawaiian whitespotted toby)	17	Benthic Carnivores	Bank	62/80	77.5%	
Canthigaster rivulata (maze toby)						
Torquigner florealis (floral puffer)						
Sebastapistes ballieui (spotfin scorpionfish)	19	Benthic Carnivores	Bank	18/19	94.7%	
Etelis carbunculus (squirrelfish snapper)	20	Benthic Carnivores	Slope	18/21	85.7%	
Lutjanus kasmira (bluestripe snapper)	21	Omnivores	Reef	14/22	63.3%	
Pristipomoides filamentosus (pink snapper)	22	Benthic Carnivores	Slope	18/24	75%	5 misclassified as 23
Pristipomoides zonatus (flower snapper)	23	Omnivores	Slope	15/19	78.9%	
Myripristis berndti (bigscale soldierfish)	24	Benthic Carnivores	Reef	16/20	80%	
Sargocentron xantherythrum (Hawaiian squirrelfish)	25	Benthic Carnivores	Bank	14/21	66.7%	
Malacanthus brevirostris (flagtail tilefish)						
Cymolutes lecluse (Hawaiian knifefish)	28	Benthic Carnivores	Bank	28/73	38.4%	7 misclassified as 17 8 misclassified as 12 6 misclassified as 11
Inistius pavo (peacock razorfish)						

Species	Top40	Diet Guild	Ecological Subsystem	Count	Proportion correctly classified (%)	Major Misclassification
Inistius umbrilatus (blackside razorfish)						
Antigonia eos (boar fish)	39	Benthic Carnivores	Subphotic	18/20	90%	
Antigonia capros (boar fish)						
Bembrops filifera (duck-billed bembropsid)	40	Benthic Carnivores	Subphotic	17/19	89.5%	

Summary of Groupings and Discriminant Analyses

While the preliminary results obtained from the discriminant analyses were promising, especially in confirming that many groups of species were reasonably distinguished from one another, the analyses also exposed several problematic issues. First, although four main clusters were clearly identified, the spatial distribution of many species groups remained clumped within these clusters. Separate analysis of each cluster generally better differentiated groups, but how this translates to differentiation in diets remains an important consideration. Second, the classification success was poor in several species groups, and in some instances, individuals for a group were consistently misclassified as others. Third, species groups represented by a small sample size could not be used in these analyses. Although other multivariate methods, including hierarchical cluster analysis, can provide insight into overall relationships among species FA signatures, we moved to model simulation procedures to better understand the degree to which prey species groups could be differentiated in diets in the QFASA model.

IV. QFASA PREY SIMULATIONS

An important feature of the QFASA model initially derived by Iverson et al. (2004) was the development of prey simulation studies. QFASA proceeds by essentially developing a mixture model of prey species FA signatures that most closely resembles that of the predator and thereby estimate its diet. A prerequisite of QFASA is an understanding of whether prey species in the database can be reliably distinguished by their FA signatures. Using simulation studies (Fig. 3), in a similar fashion to how QFASA performs actual diet estimation procedures, can be a more powerful means for assessing the reliability with which prey can be differentiated, as used in QFASA, than techniques such as DFA. Thus, while previous DFA provided some confidence in our grouping procedures and an understanding of the degree of prey differentiation achieved using small subsets of FAs in a different mathematical model, we evaluated these same groups using a complex series of simulations (see Fig. 4). These simulations aimed to basically push the limits of QFASA, by testing complex diets among a large number of species groups (40) and thus to be used as a tool to characterize among-species overlap in FA signature, allowing for species having the potential to be misclassified for one another to be identified. It has also previously been found (Iverson et al., 2004) that sequentially removing prey species that arise in diet estimates and then rerunning the model can be quite informative. The newly estimated diet can then be used to determine which species are substituted for the missing species and, therefore, allow a deeper understanding of model diet estimates.

Initial Diet Simulations

Although 65-71 FAs are routinely identified in prey and predators in the NWHI, not all provide information about diet. In our initial simulations, we used the FA subsets, based on those specified in Iverson et al. (2004), i.e., "dietary" (31 FAs) and "extended dietary" (39 FAs). The detailed procedures for performing simulations are given in Iverson et al. (2004). Briefly, the statistical software R was used to model specified species mixes, initially created using published information on monk seal potential prey species from scats and spews (Goodman-Lowe et al., 1999; Goodman-Lowe, 1998). These "pseudo diets" were computed and fitted to the QFASA model as follows:

1. A mix of 4 or 6 prey species groups, selected from the 40 species groups listed in Table 2, was specified in various proportions to make up a diet of a "pseudo seal", totalling 1.
2. For each prey group selected above, the sample of individuals was randomly split into two sets: a simulation set and a modeling set.
3. The FA signature of this pseudo seal diet was then computed from the simulation set in the proportions specified in our pseudo seal diet.
4. Next, using the modeling set of specified prey and all other prey groups in the NWHI database (Table 2), the "diet" of this pseudo seal was estimated using the QFASA model and using both the dietary and extended dietary FA sets.
5. These procedures (step 1-4) were repeated 1000 times to generate an error measure. A new split (step 2) was created every time. To strengthen interpretation of outcomes, each trial was

subsequently modeled integrating 10% noise (i.e., randomly adding prey from prey types which were not part of the diet composition vector to simulate minor random prey intake).

Results are summarized in the following tables and presented in boxplots that follow. In all plots, "a" denotes the value (proportion) specified for each of the prey species groups chosen for the diet. Each simulation was run 1000 times, and estimated diet results are represented in the box plots as the median (middle horizontal bar), the 25th (lower bar) and the 75th (top bar) percentiles of the data distribution (i.e., the box contains 50% of the data). Dots represent outliers defined as being any value greater (or less) than 1.5 times the interquartile range (75th percentile – 25th percentile) above the 75th (or below the 25th) percentile.

Trial 1

Diet composition:	no noise	w/noise
Spiny lobster	15%	13.5%
Pink snapper	15%	13.5%
Moray eel	35%	31.5%
Wrasse	35%	31.5%
noise	--	10%

Table 7.--QFASA simulation results for each of four simulation runs using the diet composition specified above. Each number represents the proportion that each species occupied in the modeled diets. Species that appeared significantly in the modeled diets but were not originally specified in the pseudo diet are also listed.

	No Noise		10% Noise	
	Dietary	Extended	Dietary	Extended
Spiny lobster	12%	10%	11.2%	9.5%
Pink snapper	5%	5%	4.6%	4.5%
Moray eel	32.2%	32.4%	29.1%	29.1%
Wrasse	19%	28%	19.9%	27.4%
Scorpionfish**	12%	7%	10.6%	5.5%
Toby**	5%	3.2%	4%	2.8%
Duckbill**	1.5%	1.7%	2%	1.9%

**species that were not included in the pseudo diet species mix

Summary:

Diet 1)   13.5-15% Spiny lobster (9-12% estimated)
           13.5-15% Pink snapper (5% estimated)
           31.5-35% Moray eel (29-32% estimated)
           31.5-35% Wrasse (19-28% estimated)

Major prey, including lobster, were reasonably picked out of all other 40 prey in the specified diet, except for pink snapper. Spiny lobster and moray eel were well-estimated, while there was some underestimation of wrasses depending on FA set. Pink snapper was especially underestimated. False positives (10% of which would be expected in the trials with noise) included primarily scorpionfish, toby and duckbill. Preliminary discriminant analysis revealed that 7% of the wrasses were consistently misclassified as scorpionfish when only dietary FAs were considered in the analysis. The current simulation results also revealed the presence of scorpionfish in the modeled diets. It is possible that the dietary FA composition of both species presents an overlap, but that overlap is greatly reduced when the extended FA list is used in the simulations. Boxplots of each run follow.

Figure 13.--Boxplot of the QFASA simulation results for diet Trial 1, using the dietary FA set, 40 species groupings, and no noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Figure 14.--Boxplot of the QFASA simulation results for diet Trial 1, using the extended dietary FA set, 40 species groupings, and no noise. 1000 iterations. The diet composition specified is represented in plots as "a".
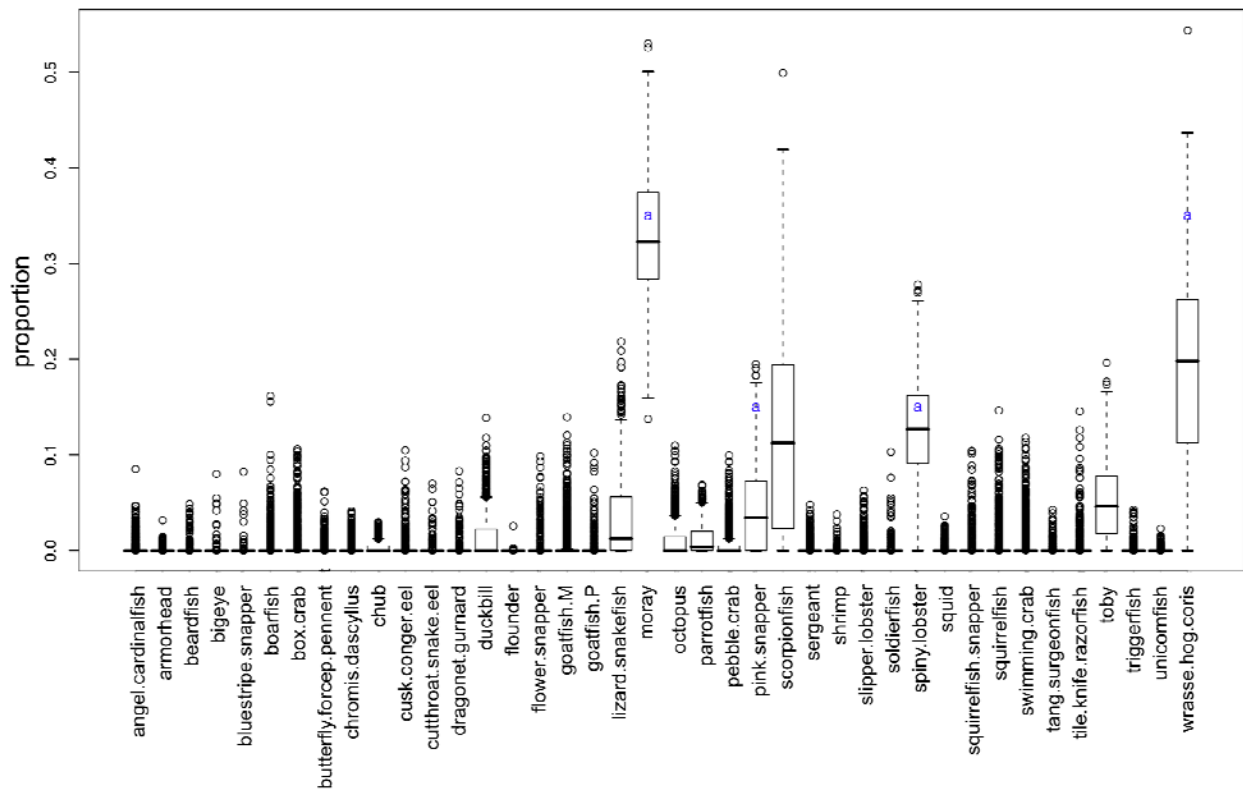

Figure 15.--Boxplot of the QFASA simulation results for diet Trial 1, using the dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Figure 16.--Boxplot of the QFASA simulation results for diet Trial 1, using the extended dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Trial 2

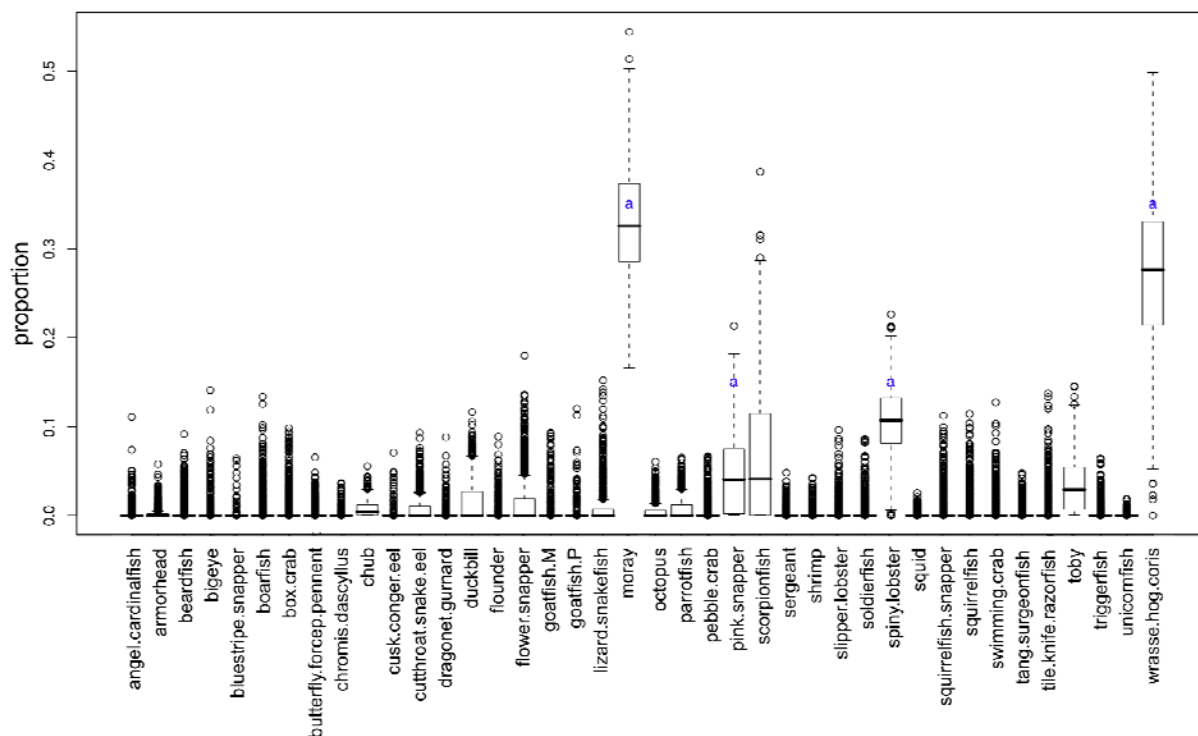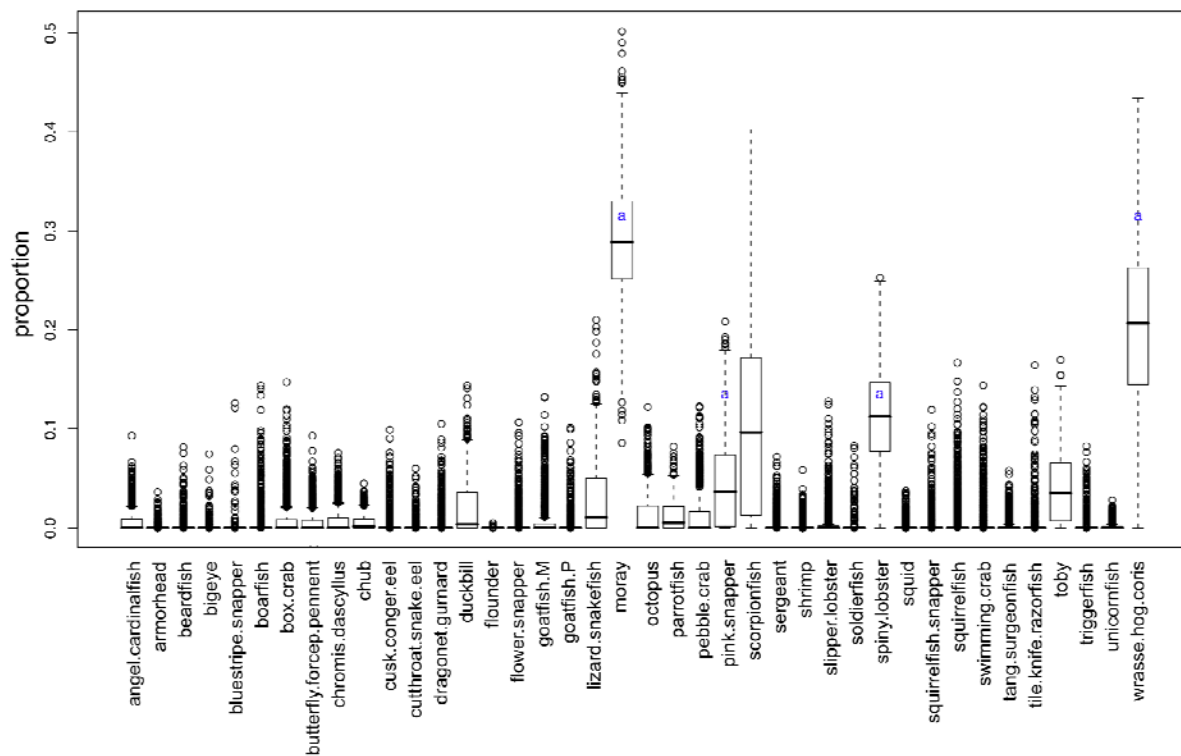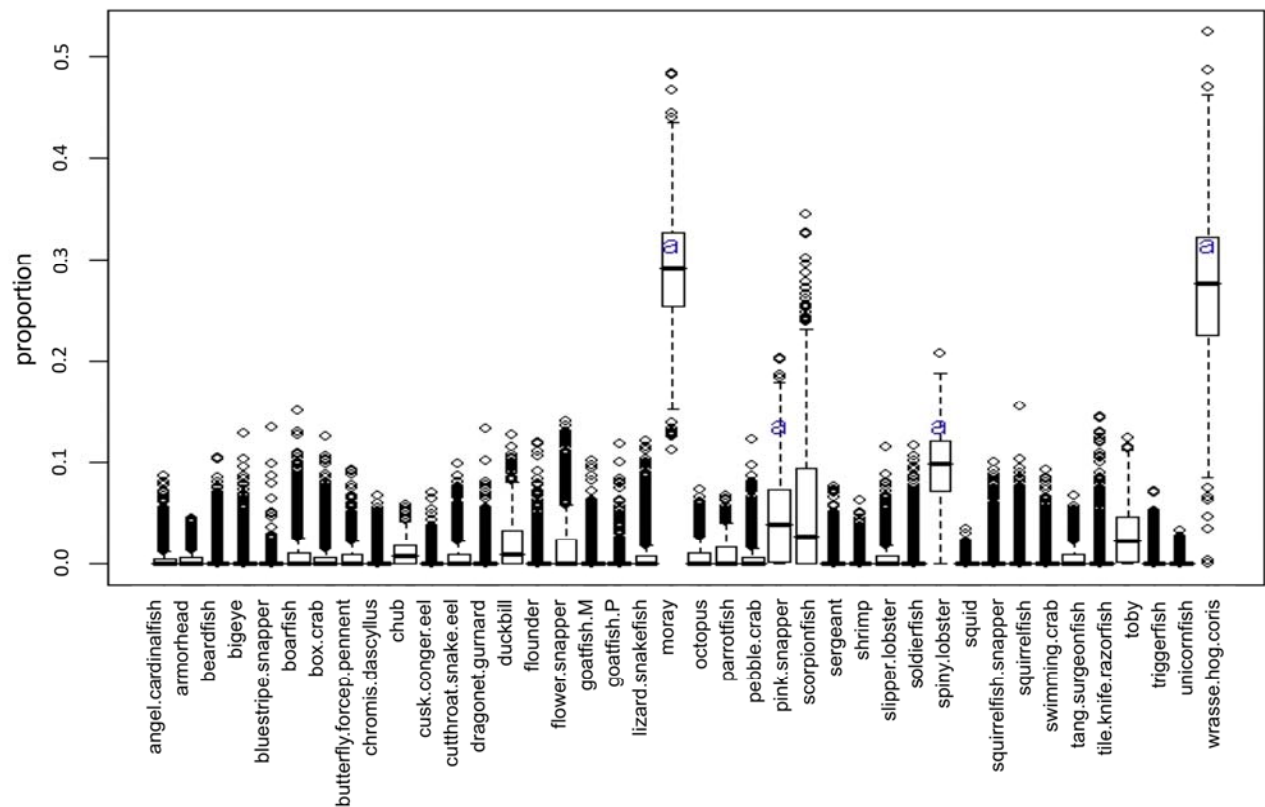| Diet Composition:	no noise	10% noise
Box crab	15%	13.5%
Cusk/conger eel	30%	27%
Flounder	15%	13.5%
Flower snapper	15%	13.5%
Spiny lobster	15%	13.5%
Squid	10%	9%
noise	--	10%

Table 8.--QFASA simulation results for each of four simulation runs using the diet composition specified above. Each number represents the proportion that each species occupied in the modeled diets. Species that appeared significantly in the modeled diets but were not originally specified in the pseudo diet are also listed.

	No Noise		10% Noise	
	Dietary	Extended	Dietary	Extended
Box crab	12.2%	15.3%	12%	14.2%
Cusk/conger eel	15.5%	15.2%	13.7%	14%
Flounder	13%	14%	11.5%	12.2%
Flower snapper	2.4%	6.7%	3%	7%
Spiny lobster	10.8%	9.8%	10.3%	9.4%
Squid	10.2%	7.9%	9.2%	7%
Squirrelfish snapper**	5.6%	4.7%	4.3%	3.9%
Duckbill**	3.5%	3.4%	3.4%	3.6%
Lizard/snakefish**	3.1%	1.4%	3.3%	1.1%
Tile/knife/razorfish**	0.7%	2%	1%	2.5%

**species that were not included in the pseudo diet species mix

Summary:

Diet 2)   13.5-15% Box crab (12-15% estimated)
              27-30% Cusk/conger eel (14-15% estimated)
              13.5-15% Flounder (12-14% estimated)
              13.5-15% Flower snapper (3-7% estimated)
              13.5-15% Spiny lobster (9-11% estimated)
              9-10% Squid (7-10% estimated)

Most of the major species, out of all 40 including crab and lobster, were well-estimated in the specified diet, except for Cusk/conger eel and flower snapper (note: snapper is problematic again). False positives included primarily squirrelfish snapper, duckbill and lizard/snakefish. The cusk/conger eel only came out as half of the proportion specified in the pseudo diet regardless of the FA set used. Preliminary discriminant analysis indicated that the classification success of conger/cusk eels on the basis of their dietary FAs composition is poor (only 36% correctly classified). The discriminant analyses also revealed that conger/cusk eels were consistently misclassified as lizard/snakefish (14% of cusk/conger eels misclassified), as morays (20% misclassified) or as tile/knife/razorfish (12% misclassified). This may explain why these three species appeared in the modeled diets.

Another problematic species are the snappers. In trial 1 (see Table 7) and in the current trial, the simulation results do not reflect the proportion of snapper originally specified in the pseudo diet. However, the classification success of the flower snapper (*Pristimoides zonatus*) was shown to be very high in previous DFA (94.7%). It is therefore possible that 1) different snapper species get misclassified for one another, which would explain the presence of the squirrelfish snapper in the modeled diet of the current trial; or 2) there is a potential overlap among the signature of snappers and other species in this ecosystem. The possibility of combining the four snapper species into one group will be examined in an upcoming trial(s), as will another method of analysis. Boxplots of each run follow.

Figure 17.--Boxplot of the QFASA simulation results for diet Trial 2, using the dietary FA set, 40 species groupings, and no noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Figure 18.--Boxplot of the QFASA simulation results for diet Trial 2, using the extended dietary FA set, 40 species groupings, and no noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Figure 19.--Boxplot of the QFASA simulation results for diet Trial 2, using the dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Figure 20.--Boxplot of the QFASA simulation results for diet Trial 2, using the extended dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Trial 3

Diet composition:

	no noise	w/noise
Parrotfish	20%	18%
Scorpionfish	15%	13.5%
Spiny lobster	15%	13.5%
Squirrelfish snapper	15%	13.5%
Triggerfish	30%	27%
Octopus	5%	4.5%
noise	--	10%

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Table 9.--QFASA simulation results for each of four simulation runs using the diet composition specified above. Each number represents the proportion that each species occupied in the modeled diets. Species that appeared significantly in the modeled diets but were originally specified in the pseudo diet are also listed.

	No Noise		10% Noise	
	Dietary FAs	Extended FAs	Dietary FAs	Extended FAs
Parrotfish	17.6%	18.3%	16.3%	17.2%
Scorpionfish	14.6%	10%	13.6%	8.6%
Spiny lobster	12.6%	10%	11.6%	9.3%
Squirrelfish snapper	2.1%	4.5%	2.2%	5%
Triggerfish	19%	27%	18%	25%
Octopus	7.5%	3.1%	6%	2.6%
Swimming crab**	5.2%	4.8%	5%	4.4%
Lizard/snakefish**	3.0%	2.1%	3%	2.2%
Moray**	2.4%	2.3%	2.9%	3%
Duckbill**	2.2%	3.7%	2.1%	3.2%
Toby**	1.6%	1.3%	1.7%	1.5%

**species that were not included in the pseudo diet species mix

Summary:

Diet 3)   18-20% Parrotfish (16-18% estimated)
             13.5-15% Scorpionfish (9-14% estimated)
             13.5-15% Spiny lobster (9-13% estimated)
             13.5-15% Squirrelfish snapper (2-5% estimated)
             27-30% Triggerfish (18-27% estimated)
             4.5-5% Octopus (3-7% estimated)

Most of the major species, out of all 40, are reasonably estimated in the specified diet. Four of the six species specified in the pseudo diet of Trial 3 (i.e., parrotfish, scorpionfish, spiny lobster, and triggerfish) were well represented in the modeled diets; the specified proportions of each species were almost the same in the pseudo and modeled diets. There was some underestimation of triggerfish depending on FA set used. However, once again snapper, this time squirrelfish snapper, was quite underestimated. False positives included primarily swimming crab, lizard/snakefish, moray eel, toby and duckbill. Boxplots of each run follow.
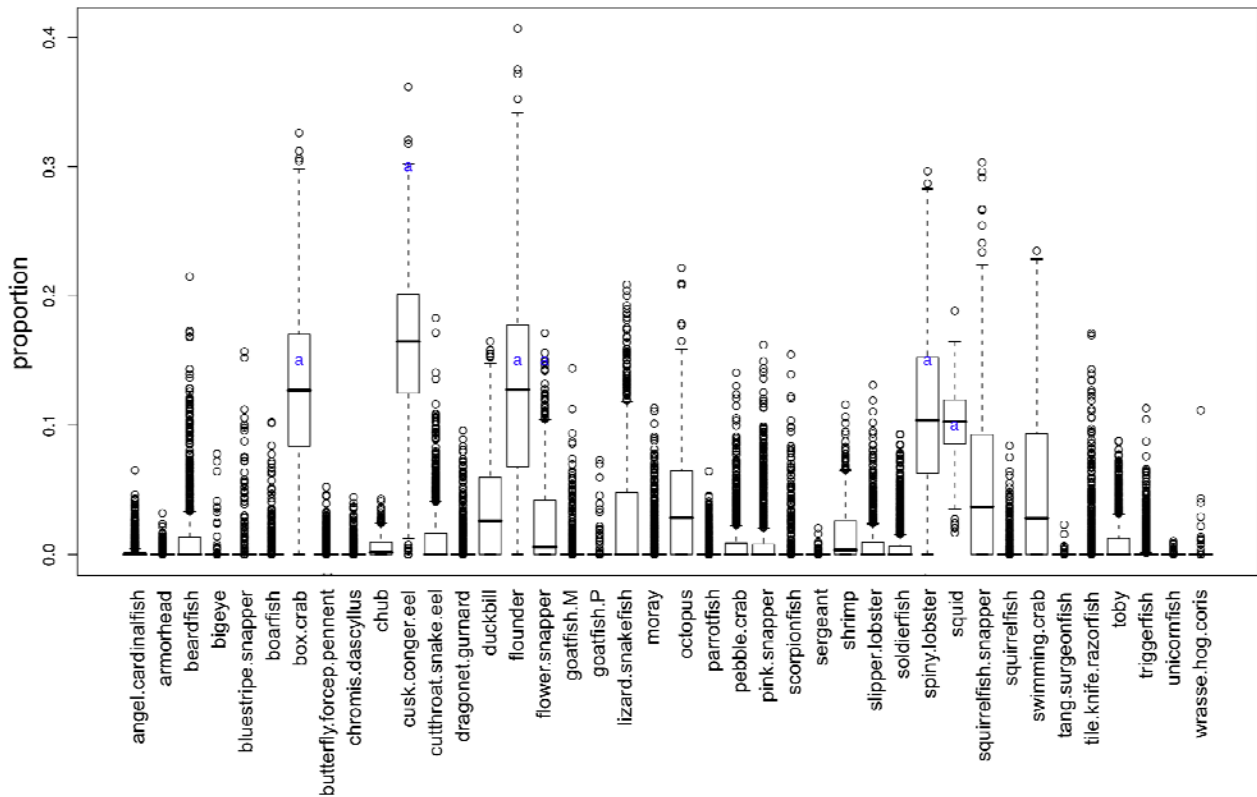
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Figure 21.--Boxplot of the QFASA simulation results for diet Trial 3, using the dietary FA set, 40 species groupings, and no noise. 1000 iterations. The diet composition specified is represented in plots as "a".
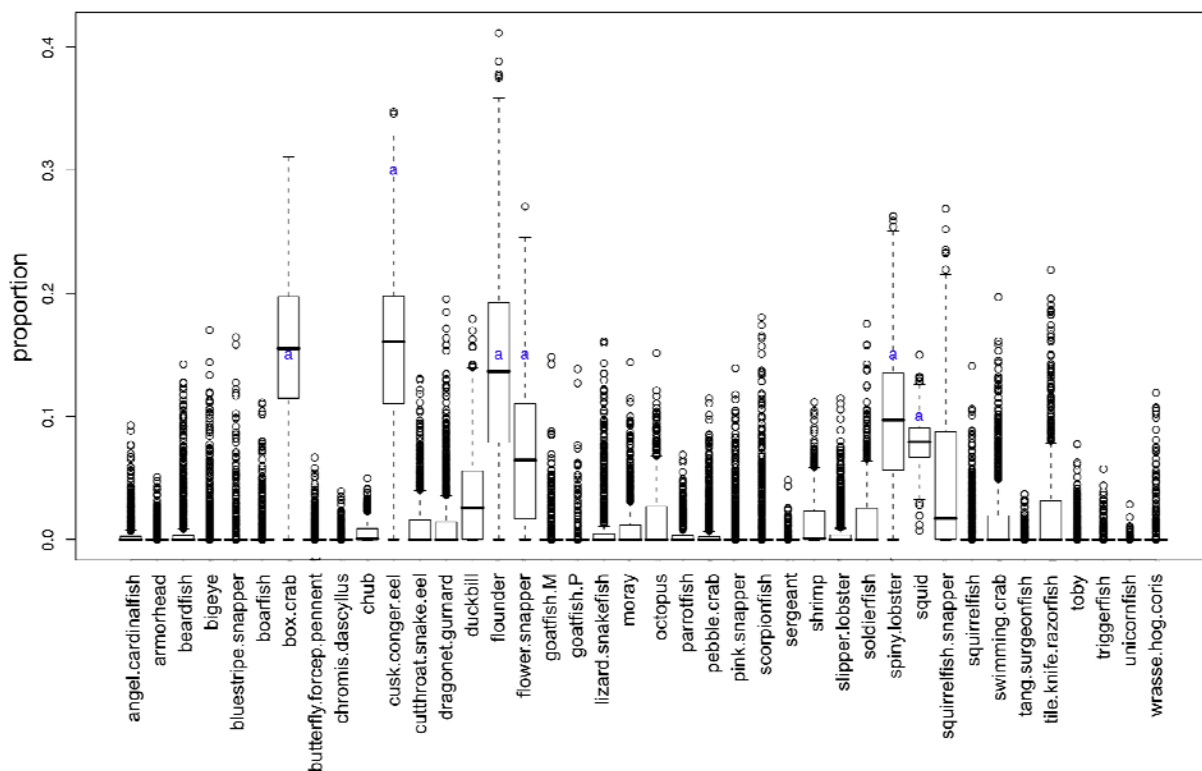

Figure 22.--Boxplot of the QFASA simulation results for diet Trial 3, using the extended dietary FA set, 40 species groupings, and no noise. 1000 iterations. The diet composition specified is represented in plots as "a".
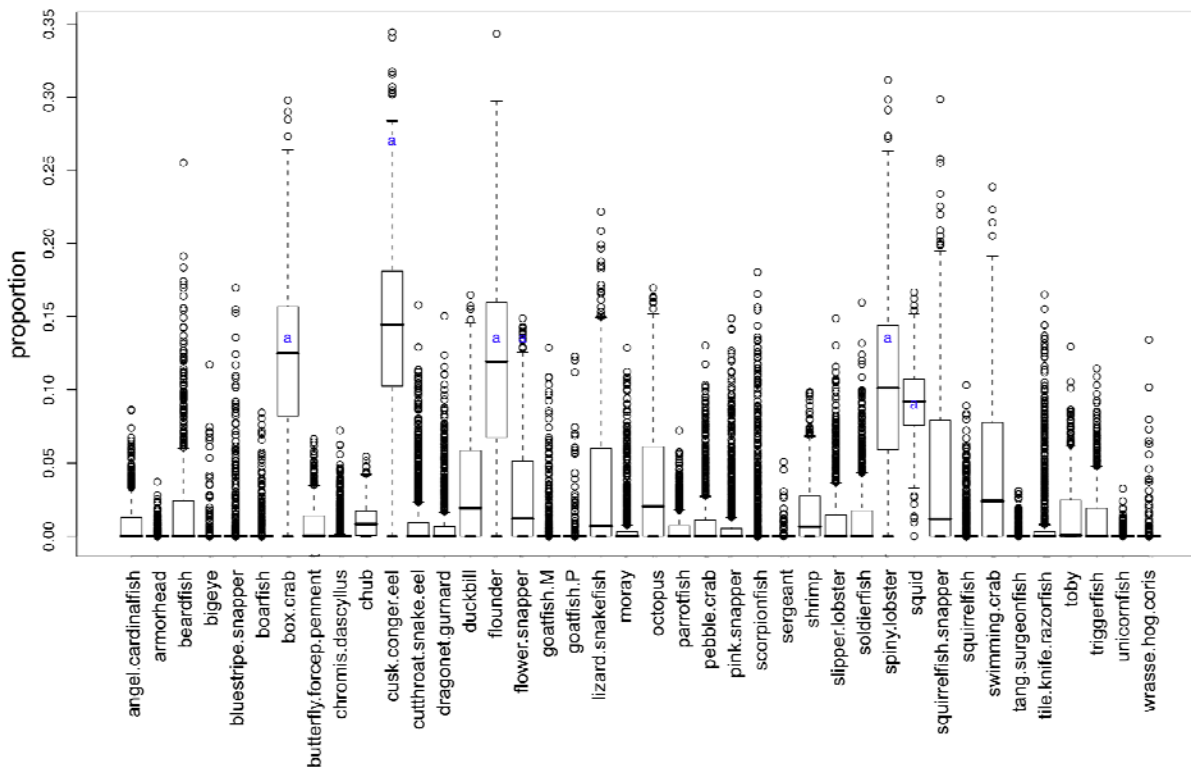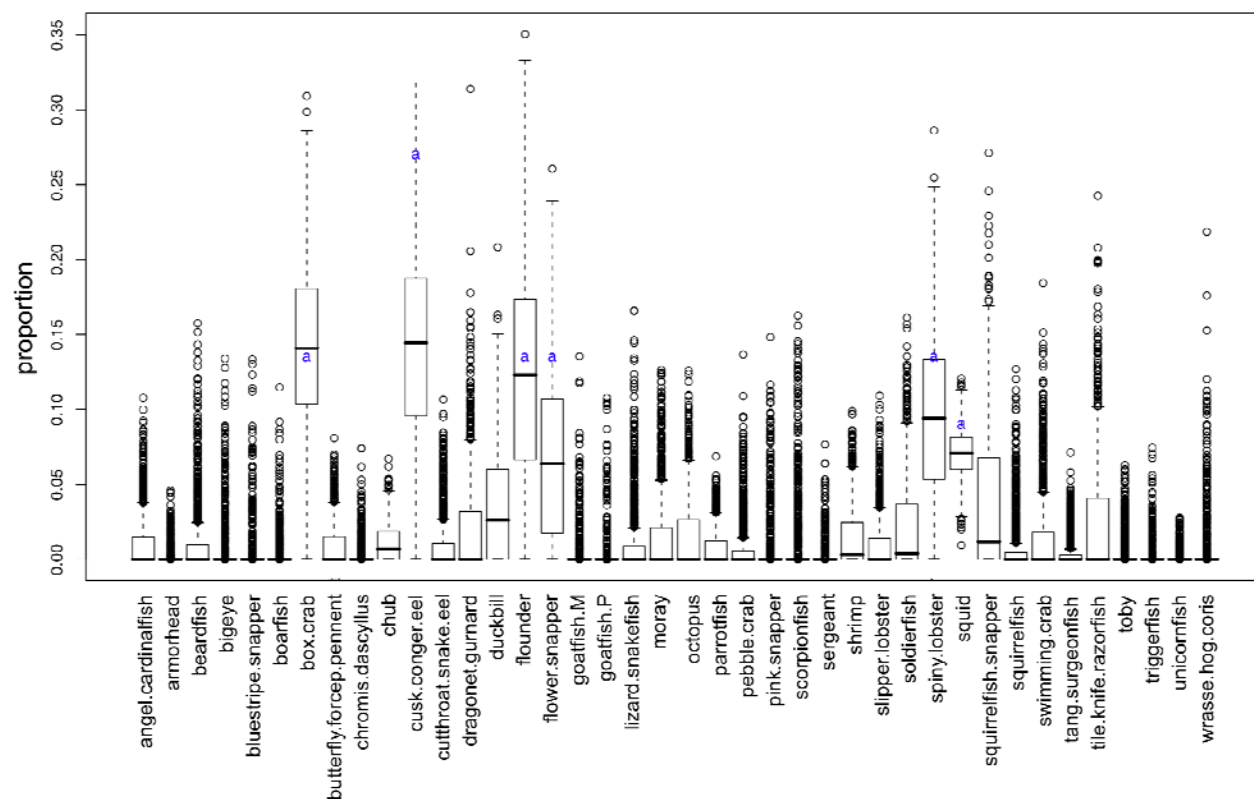
Figure 23.--Boxplot of the QFASA simulation results for diet Trial 3, using the dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".
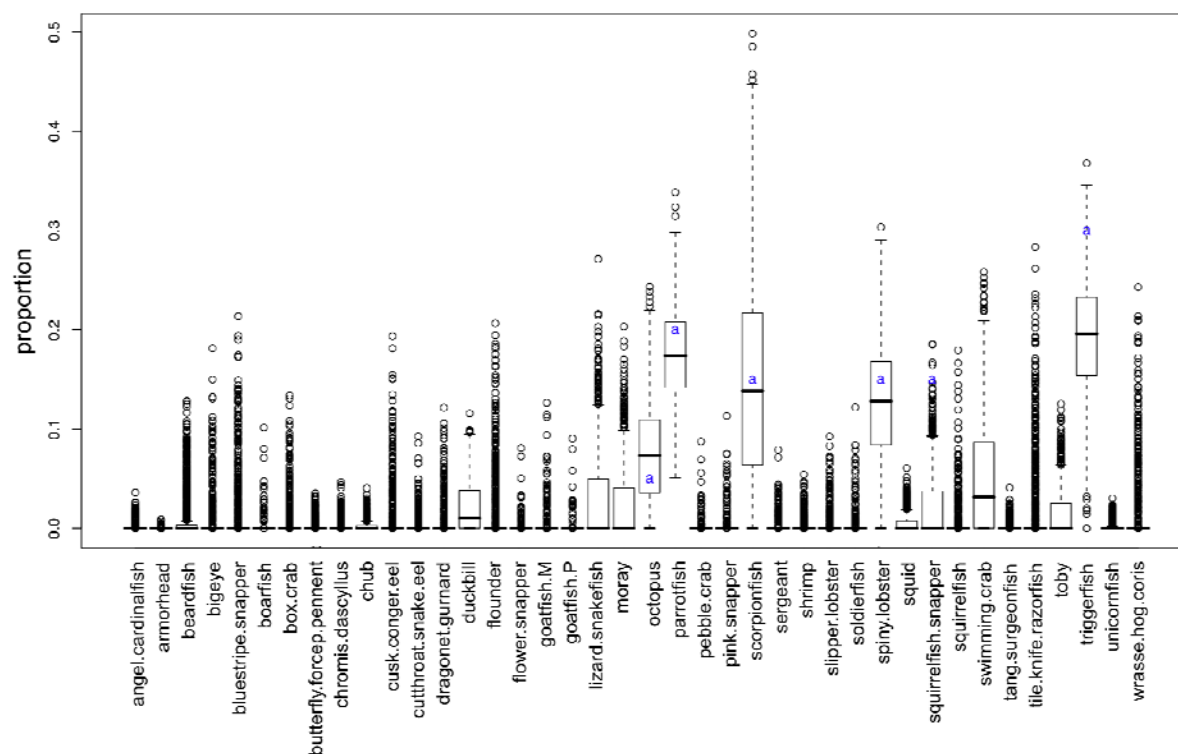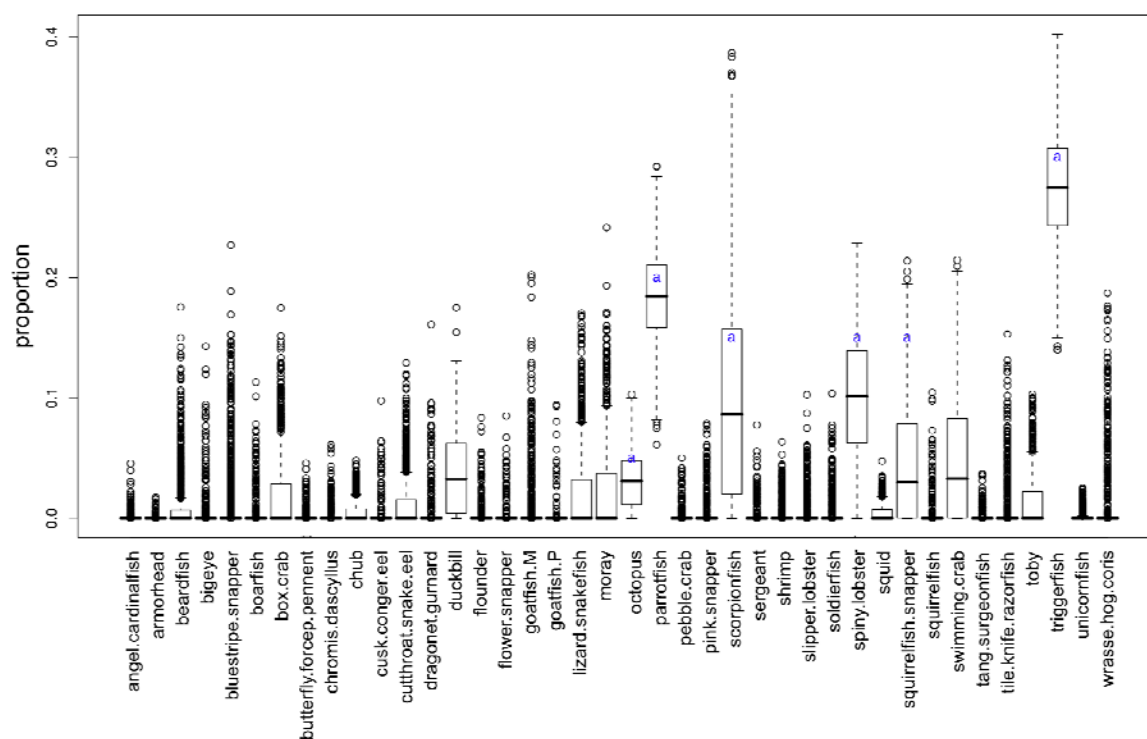

Figure 24.--Boxplot of the QFASA simulation results for diet Trial 3, using the extended dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Trial 4

Diet Composition:		no noise	w/noise
Angel/cardinalfish		15%	13.5%
Goatfish M		35%	31.5%
Bluestripe snapper		35%	31.5%
Pebble crab		15%	13.5%
noise	--		10%

Table 10.--QFASA simulation results for each of four simulation runs using the diet composition specified above. Each number represents the proportion that each species occupied in the modeled diets. Species that appeared significantly in the modeled diets but were not originally specified in the pseudo diet are also listed.

	No Noise		10% Noise	
	Dietary	Extended	Dietary	Extended
Angel/cardinalfish	10%	12.4%	10%	13%
Goatfish M.	20%	25%	18.5%	23%
Bluestripe snapper	3%	8.5%	3%	7.4%
Pebble crab	9.6%	8.1%	9.3%	7.9%
Tile/knife/razorfish**	9.4%	2.7%	8.9%	2.8%
Goatfish P.**	7.2%	4.4%	7.2%	4.4%
Bigeye**	4.2%	2.3%	3.4%	2.2%
Dragonet/gurnard**	3%	4.5%	3.4%	3.8%
Flounder**	4.5%	0.5%	3.9%	0.6%
Slipper lobster**	2.8%	3.0%	2.8%	2.9%
Boarfish**	2.4%	2.7%	2.7%	3.1%
Shrimp**	2.6%	2.5%	2.6%	2.5%

**species that were not included in the pseudo diet species mix

Summary:

Diet 4)   13.5-15% Angel/cardinalfish (10-13% estimated)
             31.5-35% Goatfish M (20-25% estimated)
             31.5-35% Bluestripe snapper (3-9% estimated)
             13.5-15% Pebble crab (8-10% estimated)

Angel/cardinalfish and pebble crab are well estimated, out of all 40, in the specified diet, although goatfish M. were underestimated. Again, snapper, this time bluestripe snapper was very significantly underestimated. False positives included primarily goatfish p. and tile/razor fish group, plus other more minor items (a bit of pebble crab may go to another crab/lobster). Boxplots of each run follow.
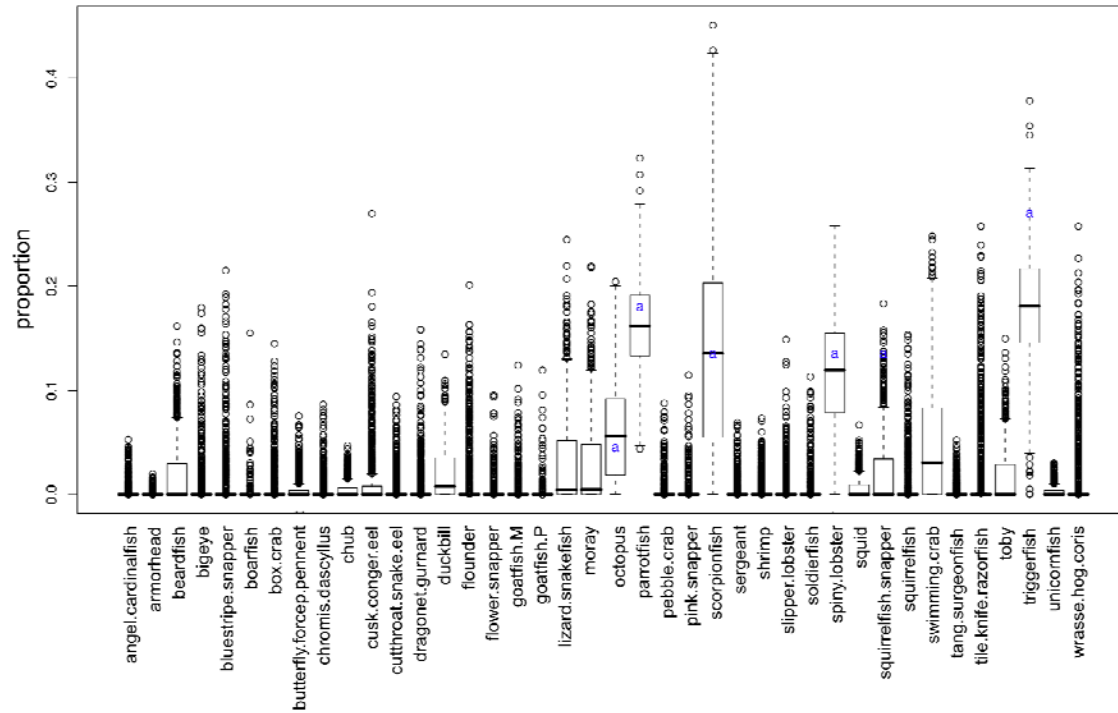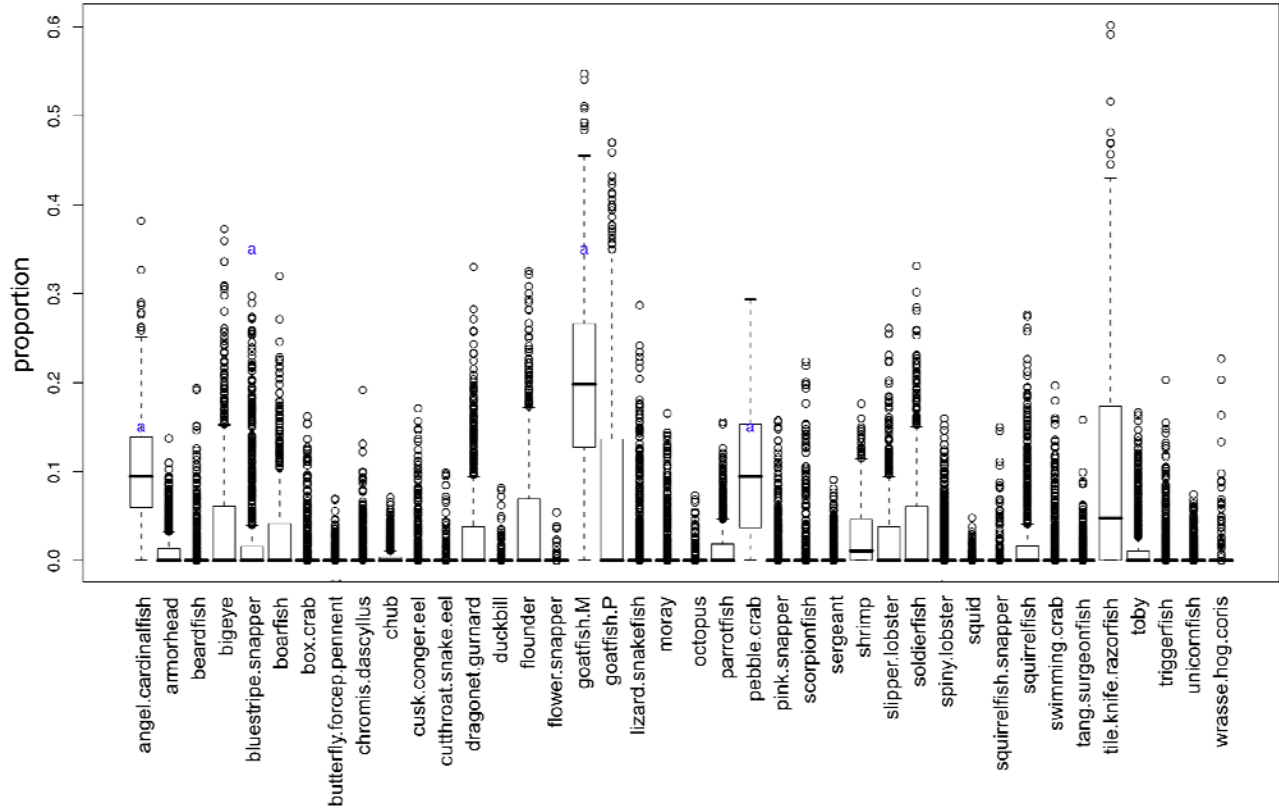

Figure 25.--Boxplot of the QFASA simulation results for diet Trial 4, using the dietary FA set, 40 species groupings, and no noise. 1000 iterations. The diet composition specified is represented in plots as "a".
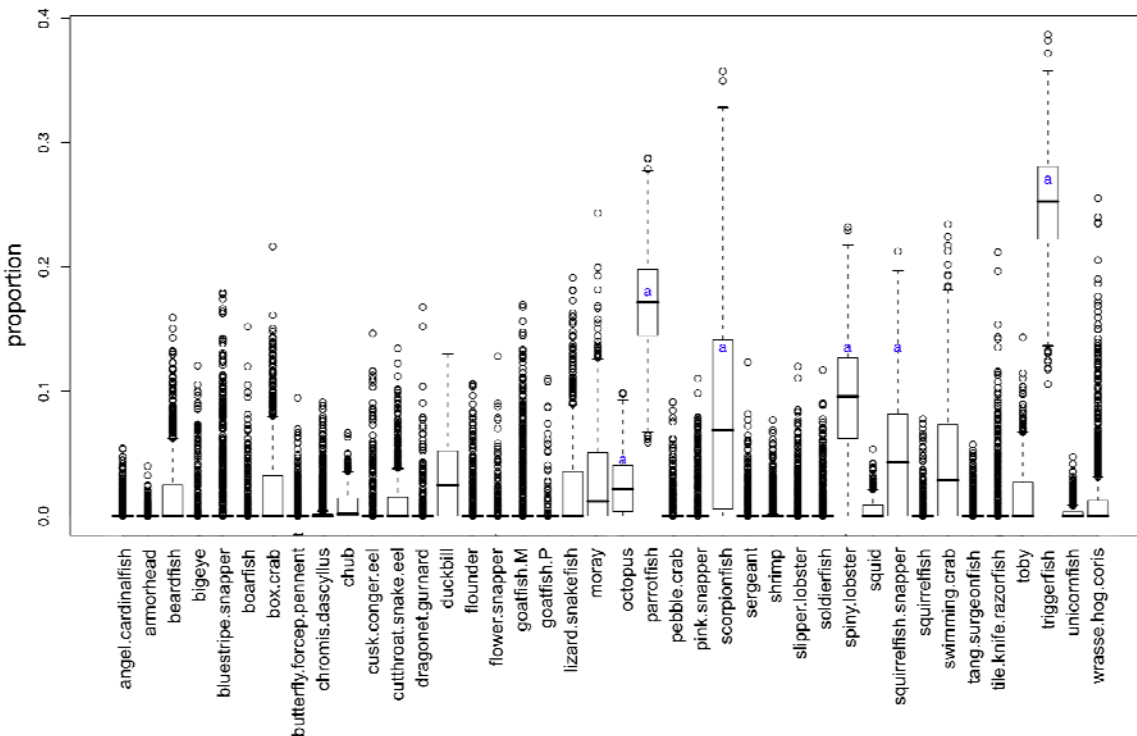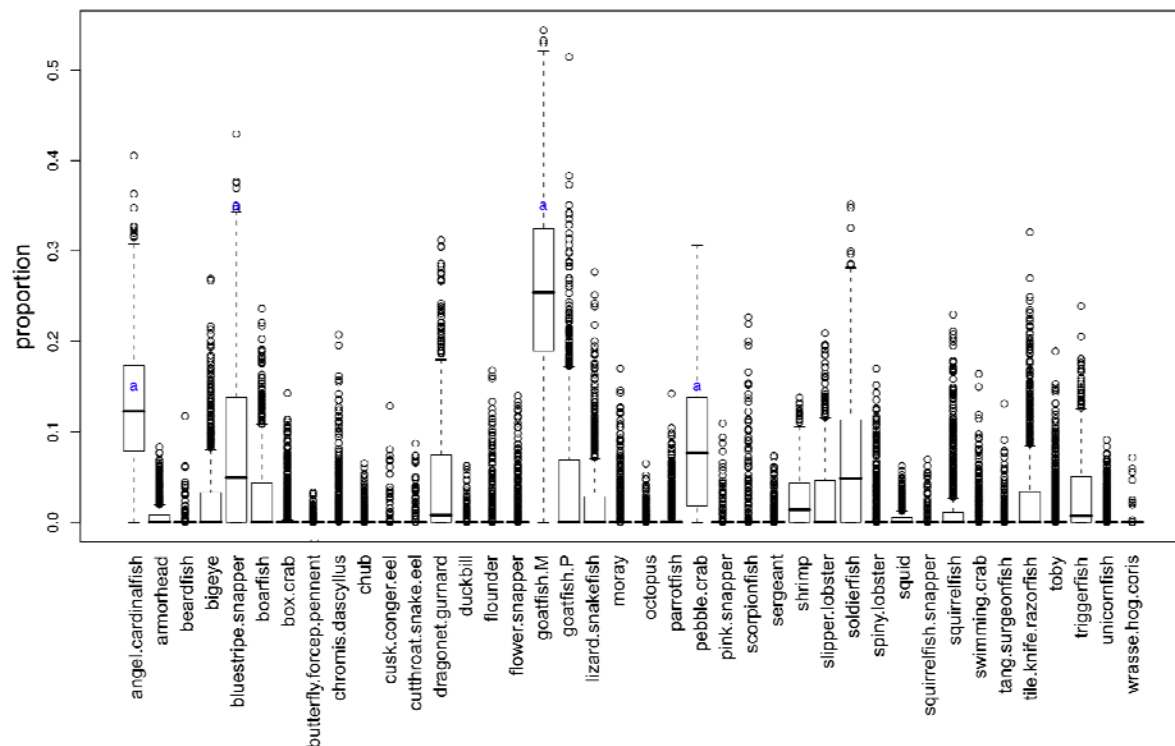
Figure 26.--Boxplot of the QFASA simulation results for diet Trial 4, using the extended dietary FA set, 40 species groupings, and no noise. 1000 iterations. The diet composition specified is represented in plots as "a".
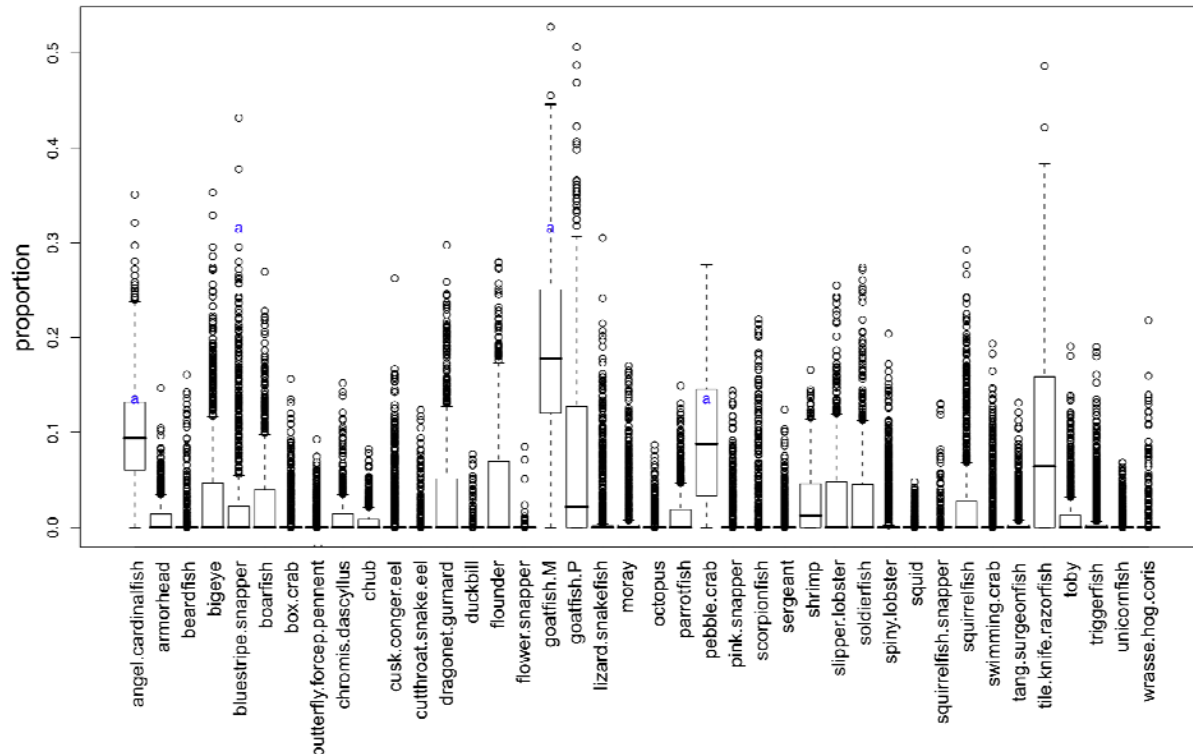
Figure 27.--Boxplot of the QFASA simulation results for diet Trial 4, using the dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".
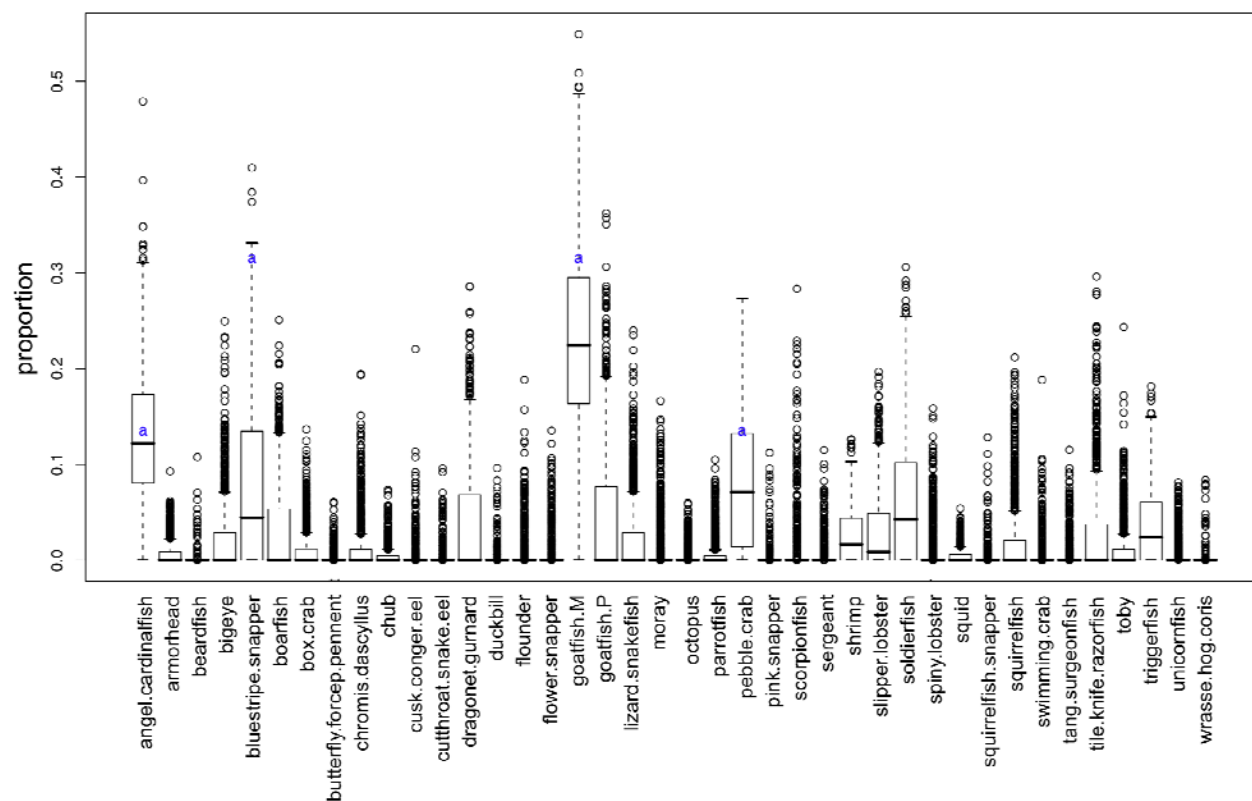
Figure 28.--Boxplot of the QFASA simulation results for diet Trial 4, using the extended dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Trial 5

Diet Composition:	no noise	w/noise
Butterfly/forcep/pennantfish	20%	18%
Chromis/dascyllus	20%	18%
Duckbill	15%	13.5%
Lizard/snakefish	15%	13.5%
Slipper lobster	20%	18%
Swimming crab	10%	9%
noise	--	10%

Table 11.--QFASA simulation results for each of four simulation runs using the diet composition specified above. Each number represents the proportion that each species occupied in the modeled diets. Species that appeared significantly in the modeled diets but were not part of the pseudo diet are also listed.

	No Noise		10% Noise	
	Dietary	Extended	Dietary	Extended
Butterfly/forcep/pennantfish	12.6%	15.8%	12%	15%
Chromis/dascyllus	9.6%	9.6%	9.3%	9.4%
Duckbill	9.3%	9.1%	9.5%	9.4%
Lizard/snakefish	15.2%	12.4%	14%	11.3%
Slipper lobster	7.8%	10.9%	7.9%	10%
Swimming crab	1.3%	3.2%	1.4%	3%
Spiny lobster**	9.7%	4.4%	8.9%	4.8%
Scorpionfish**	3.6%	3.3%	4%	3.3%
Bigeye**	3.4%	3.3%	3.1%	3.6%
Pebble crab**	2.8%	3.1%	2.4%	2.6%
Tang/surgeonfish**	2.8%	2.5%	2.8%	2.7%
Soldierfish**	1.9%	1.2%	1.6%	1.2%
Sergeant**	1.8%	3.1%	2.1%	3.4%
Moray**	1.3%	0.5%	1.5%	0.8%
Triggerfish**	1%	1.8%	1.1%	1.9%

**species that were not included in the pseudo diet mix

Summary:

Diet 5)   18-20% Butterfly/forcep/pennantfish (12-16% estimated)
           18-20% Chromis/dascyllus (9-10% estimated)
           13.5-15% Duckbill (9-10% estimated)
           13.5-15% Lizard/snakefish (11-15% estimated)
           18-20% Slipper lobster (8-11% estimated)
           9-10% Swimming crab (1-3% estimated)

Simulations generally performed the worst on this diet, with some underestimation of all prey specified. Nevertheless, the specified species still were the ones estimated to make up the major portion of the diet, except for much slipper lobster and swimming crab coming in as spiny lobster and pebble crab. (Boxplots are not illustrated.)

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Second Diet Simulations: Combining Problematic Species Groups

Snappers

The previous simulations allowed for several problematic species to be identified. First, simulation results (Trials 1-4) indicated that the four species of snappers (*E. carbunculus, L. kasmira, P. filamentosus, P. zonatus*) represented at the most 50% of the proportion they were expected to occupy in their respective trial, relative to the proportions specified in each prey species mix. This could be explained by results of previous discriminant analyses, which indicated that some snapper species in the NWHI exhibit similarities at the level of their FA signatures – in fact all the 3 deepwater snappers clustered closely together. For example, it was demonstrated that 25% of the pink snappers were consistently misclassified as flower snappers. In order to determine whether the four snapper species should be grouped together for further modeling, their respective FA signatures were plotted (Fig. 29). It was expected that the bluestripe snapper *L. kasmira*, a shallow-water species, would be distinguishable from the three deepwater snapper species. However, all snappers appeared to have somewhat similar FA signatures when plotted on the extended dietary set of FAs. The four species were, therefore, combined and considered as one in this series of simulations.

Goatfishes

Two genus of goatfishes (*Mulloidichthys* and *Parupeneus* spp.) were originally considered as two different species groups among the 40 groups used in the modeling process. However, classification results of previous discriminant analyses have revealed that goatfishes of the two genuses can be consistently misidentified as one another. In addition, the results of Trial 4 (both goatfish groups appeared in the modeled diets while only goatfish M. was specified in the pseudo diet) suggested that there might be an overlap in the FA signatures of the two groups of goatfishes. The FA signatures of both species groupings were plotted to check for commonalities in patterns. As depicted in Figure 30, the FA signatures of these two genuses of goatfishes was similar when plotted on the extended dietary FA set. The goatfishes were, therefore, combined and treated as a single species for the following sets of simulations.
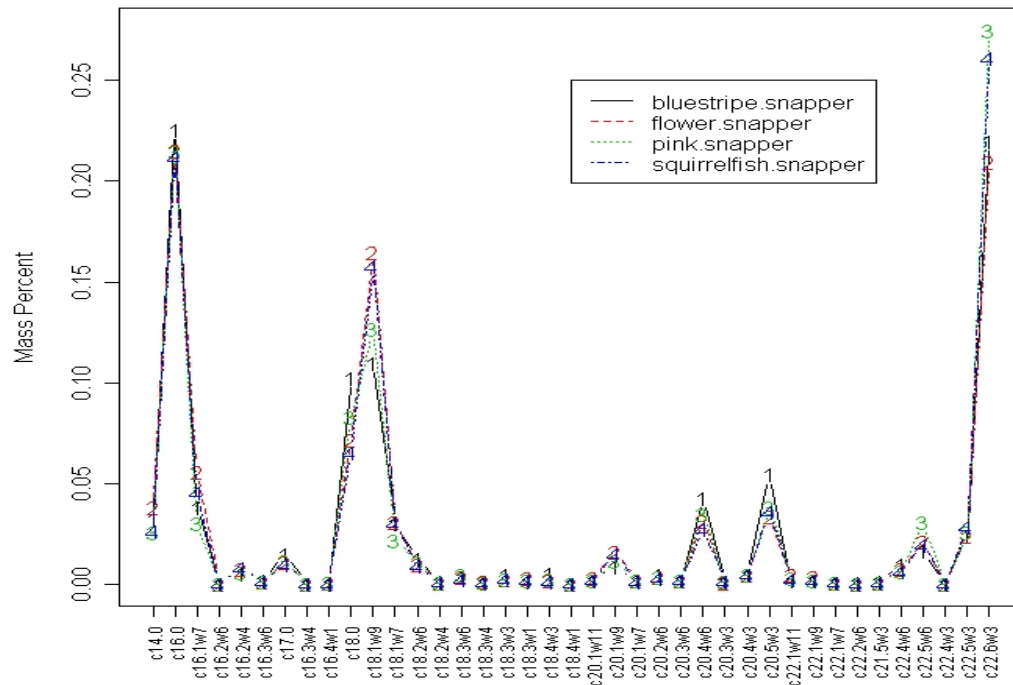
Figure 29.--Plot of the FA signatures (using the extended dietary set) of the four species of snappers. The bluestripe snapper is a shallow-water species, while the three other species are deepwater snappers. Each peak represents the proportion of the total FA signature occupied by the corresponding FA (x axis).
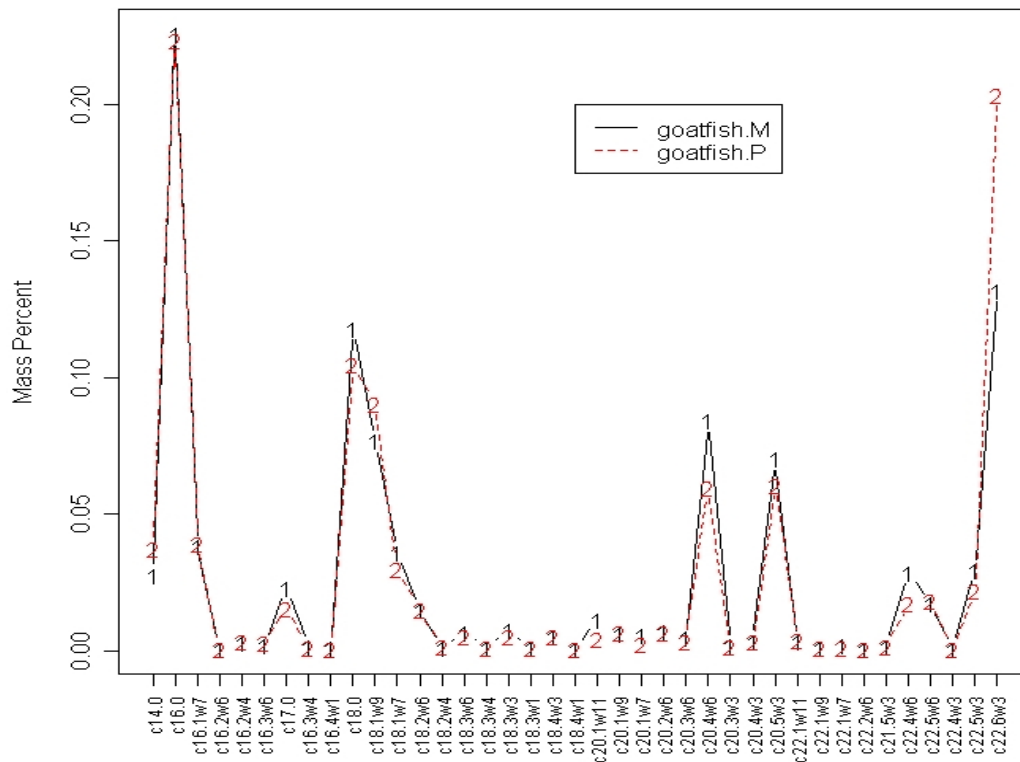
Figure 30.--Plot of the FA signatures (using the extended dietary set) of the two genuses (goatfish M. = *Mulloidichthys* spp., goatfish P. = *Parupeneus* species) of goatfish. Each peak represents the proportion of the total FA signature occupied by the corresponding FA (x axis).

Trial 6: Combined Species (snappers)

Diet Composition:	no noise	w/noise
Angel/cardinalfish	15%	13.5%
Snapper	35%	31.5%
Goatfish	35%	31.5%
Moray eel	15%	13.5%
noise	--	10%

Table 12.--QFASA simulation results for each of four simulation runs using the diet composition specified above. Each number represents the proportion that each species occupied in the modeled diets. Species that appeared significantly in the modeled diets but were not originally included in the pseudo diet are also listed.

	No Noise		10% Noise	
	Dietary	Extended	Dietary	Extended
Angel/cardinalfish	9.1%	11.7%	9.1%	11.8%%
Snapper	12.3%	16.9%	10.9%	14.5%
Goatfish	14.8%	17%	12.2%	14.4%
Moray	12.4%	13.2%	12.5%	13.5%
Lizard/snakefish**	7.5%	1.9%	7.4%	2%
Boarfish**	7.2%	3.8%	5.4%	3.3%
Dragonet/gurnard**	6.7%	5.6%	6.7%	5.6%
Squirrelfish**	5%	2.6%	4.9%	2.5%
Tile/knife/razorfish**	4.8%	2.9%	5.1%	3.2%
Soldierfish**	0.9%	4.2%	0.9%	4%
Bigeye**	3%	3.7%	2.8%	3.2%
Cusk/conger eel**	3%	2.5%	3.3%	2.7%
Toby**	2.1%	2%	2.5%	2.2%
Duckbill**	1.8%	3.4%	1.8%	3.6%

**species that were not included in the pseudo diet mix

Summary:

Diet 6)　　　　13.5-15% Angel/cardinalfish (9-12% estimated)
　　　　　　　31.5-35% Snapper (11-17% estimated)
　　　　　　　31.5-35% Goatfish (12-17% estimated)
　　　　　　　13.5-15% Moray eel (12-14% estimated)

Major prey estimated in the diet were those specified, but although angel/cardinalfish and moray eels were well-estimated (e.g., Fig. 31), snappers and goatfish were estimated at only 50% of that specified, suggesting overlap with other species.

Figure 31.--Boxplot of the QFASA simulation results for diet Trial 6, using the extended dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".

Trial 7: Combined Groups (goatfishes)

The following pseudo diet was created in an attempt to identify which species the snappers were consistently mistaken for.

Diet Composition:	no noise	w/noise
Snapper	90%	81%
Moray	10%	9%
noise	--	10%

Table 13.--QFASA simulation results for each of four simulation runs using the diet composition specified above. Each number represents the proportion that each species occupied in the modeled diets. Species that appeared significantly in the modeled diets but were not originally specified in the pseudo diet are also listed.

	No Noise		10% Noise	
	Dietary	Extended	Dietary	Extended
Snapper	66.2%	66.2%	52.9%	57.6%
Moray	7.6%	8%	8.8%	9.2%
Lizard/snakefish**	8.8%	4.9%	9.9%	3.8%
Duckbill**	5.4%	6.3%	4.9%	6%
Boarfish**	4.3%	2.3%	5.2%	2.6%
Beardfish**	2.3%	2.2%	3.5%	2%

**species that were not included in the pseudo diet mix

Summary:

Diet 7)   81-90% Snapper (53-66% estimated)
               9-10% Moray (8-9% estimated)

Moray eels were well-estimated in diets. Although snapper was better estimated than previously, it remained underestimated by 25% of that specified (e.g., Fig. 32). Duckbill, boarfish and beardfish appear to remain among those groups that are frequently mistakenly identified as species in the diet (see Trials 1, 2, 3, 4, 6), along with lizard/snakefish (*see* Trials 2, 3, 6).
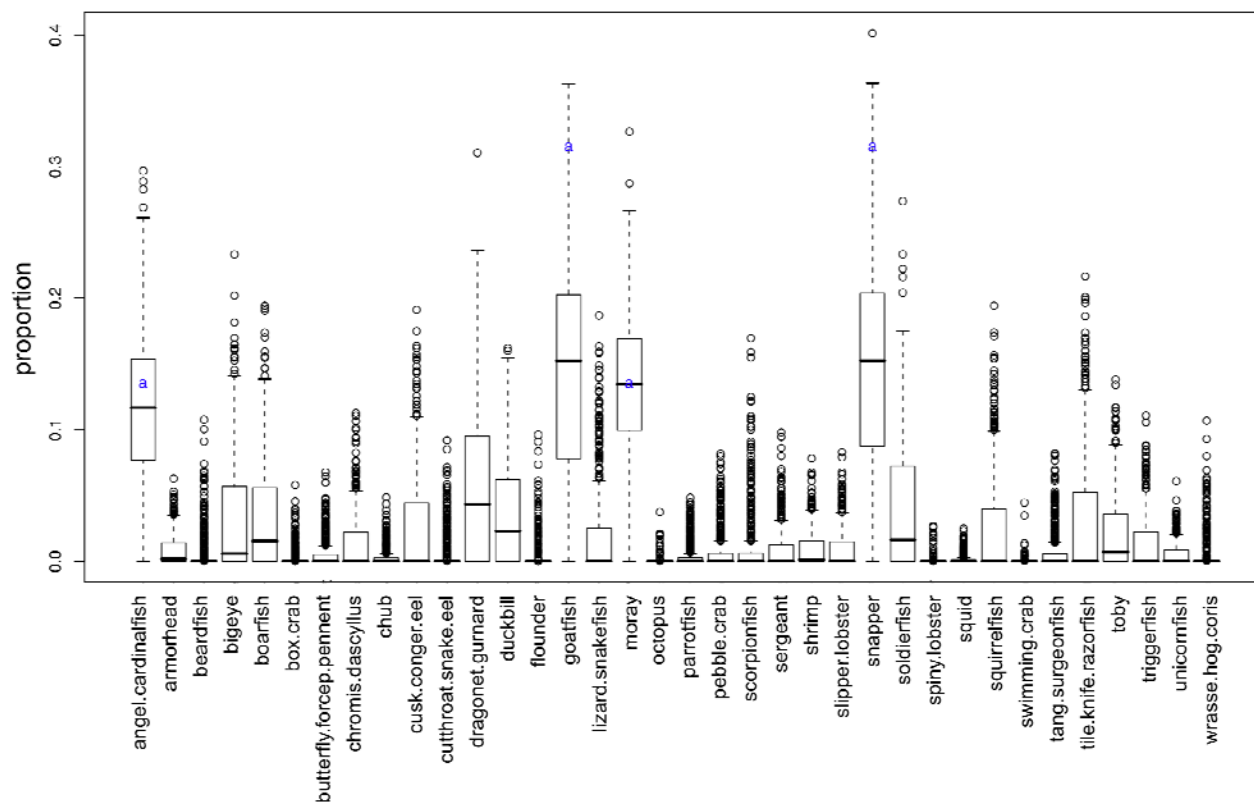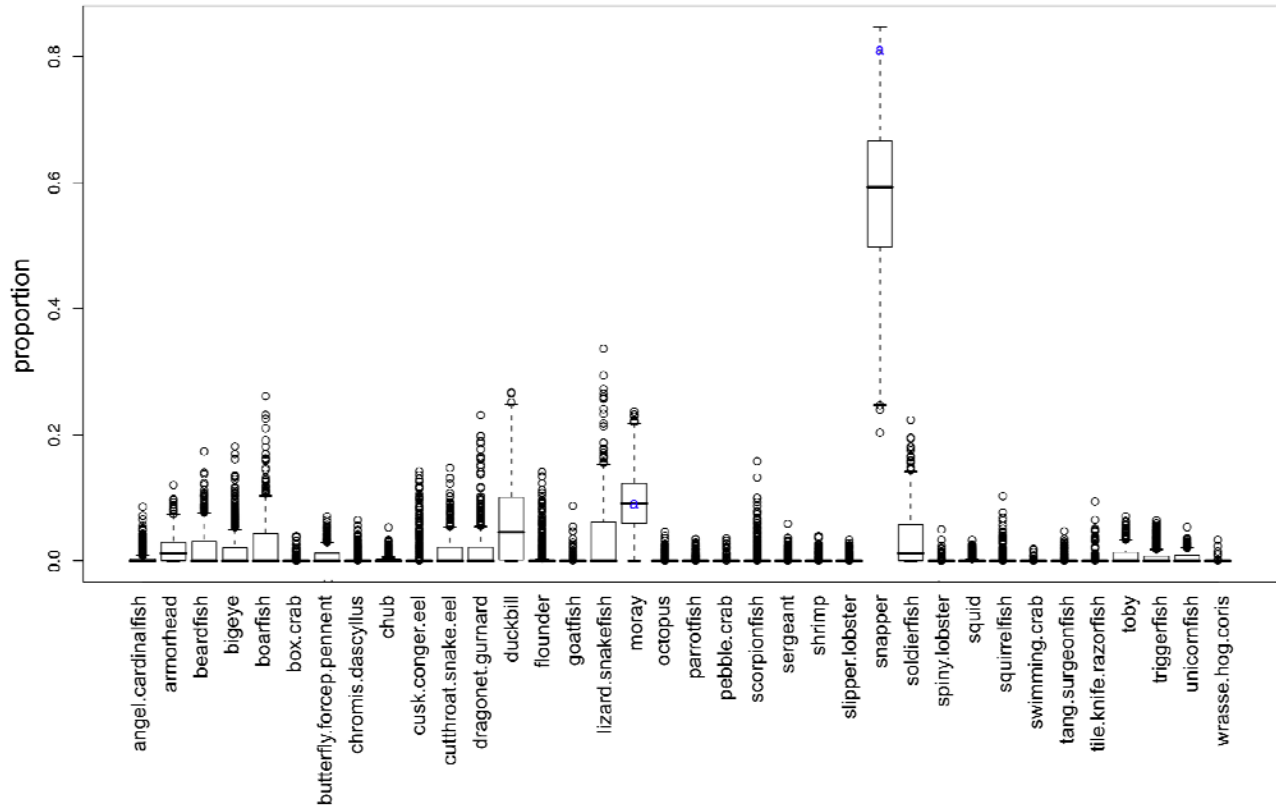
Figure 32.--Boxplot of the QFASA simulation results for diet Trial 7, using the extended dietary FA set, 40 species groupings, and 10% noise. 1000 iterations. The diet composition specified is represented in plots as "a".

The model appeared to perform better when combining snappers in this trial. However, when diet Trial 4 (see above) was modeled using the combined snappers and goatfish, the model actually performed worse. Thus, a simple combining of snappers, or other groups might not be the best answer (see following section). For instance, it may perhaps be better to model snappers separately but to understand that especially the deepwater snappers may have overlap with one another.

Third Diet Simulations: Evaluation of FA Sets, Removing False Positives, and Modeling 10 Species of Interest

Evaluation of FA Sets

The first two series of simulations were conducted on two sets of FAs (dietary and extended dietary) and yielded fairly promising results with the overall detection of major prey species in simulated diets out of all 40 groups. However, while the absolute proportions of species groups in the modeled diet were sometimes quite similar to the proportions specified in the pseudo diet, some species groups, especially snappers, were underestimated, with other species groups not included in the pseudo diets erroneously appearing as false positives. Although these would be expected in all simulations that included 10% noise, their presence was higher than specified. In

an attempt to further determine what influence the set of FAs had on the model and which would yield the best simulation results, two new sets of FAs were employed to run supplementary analyses (see Appendix C). A third set of FAs for modeling ('modified 1 extended dietary') was created largely following work on captive Steller sea lions and harbor seals (S. Iverson and D. Tollit, unpublished data), which had measured equally reliable and comparable calibration (CC) estimates to those of monk seals. The fourth set of FAs ('modified 2 extended dietary'), was constructed from the extended dietary list, but where FAs with an average mean of less than 0.10% mass percent, and FAs that were routinely zeros in many NWHI prey species, were removed from the list.

The following two re-simulations and subsequent removal of prey, demonstrate the kind of iterations that can be performed, which result in high classification success and species identification in simulated diets.

Trial 1.2

Diet composition (from the original Trial 1): 15% Spiny lobster, 15% Pink snapper, 35% Moray eel, and 35% Wrasse

Table 14.--Results of QFASA simulations conducted using the pseudo diet composition of Trial 1 modeled on the modified 1 and 2 extended dietary FA sets. The results of the previous simulations conducted using the dietary and extended dietary FA set are displayed for comparison. Two simulations were run for each set (with or without 10% noise). The total percent false positive is presented, which is a measure of the total proportion of species that have been misclassified. Also listed are the major false positives groups that appeared in the modeled diets.

	No Noise				10% Noise			
	Dietary	Extended	Modified 1	Modified 2	Dietary	Extended	Modified 1	Modified 2
Spiny lobster (15/13.5%)	12%	10%	11.6%	10.8%	11.2%	9.5%	10.5%	9.8%
Pink snapper (15/13.5%)	5%	5%	5.7%	5.1%	4.6%	4.5%	5.4%	4.8%
Moray eel (35/31.5%)	32.2%	32.4%	34.2%	33.3%	29.1%	29.1%	30.0%	29.2%
Wrasse (35/31.5%)	19%	28%	26.4%	26.8%	19.9%	27.4%	26.6%	27.2%
Scorpion fish **	12%	7%	4.9%	5.7%	10.6%	5.5%	4.5%	5.2%
Toby**	5%	3.2%	3.0%	3.3%	4%	2.8%	2.7%	2.8%
Duckbill**	1.5%	1.7%	1.6%	1.7%	2%	1.9%	1.7%	1.7%
Others**	13.3%	12.7%	12.6%	13.3%	18.6%	19.3%	18.6%	19.3%
% False Positive	31.8%	26.60%	22.1%	24.0%	35.2% (25.2%)	29.5% (19.5%)	27.5% (17.5%)	29.0% (19.0%)

**Species that were not included in the pseudo diet species mix. Only the unspecified species representing more than 1.5% of the modeled diet are listed in the table. The rest are grouped as a single 'others' category.
Boxes highlight the best results (i.e., the lowest proportion of false positives) for the no noise simulation.

Trial 3.2

Diet composition (from the original Trial 3): 20% Parrotfish, 15% Scorpionfish, 15% Spiny lobster, 15% Squirrelfish snapper, 30% Triggerfish, and 5% Octopus.

Table 15.--Results of simulations conducted using the pseudo diet composition of Trial 3 modeled on the modified 1 and 2 extended dietary sets FA. The results of the previous simulations conducted using the dietary and extended dietary FA set are displayed for comparison. Two simulations were run for each set (with or without 10% noise). The percent false positive is presented, which is a measure of the total proportion of species that have been misclassified. Also listed are the major false positive groups that appeared in the modeled diets.

	No Noise				10% Noise			
	Dietary	Extended	Modified 1	Modified 2	Dietary	Extended	Modified 1	Modified 2
Parrotfish (20/18%)	17.6%	18.3%	18.2%	18.4%	16.3%	17.2%	16.9%	17.1%
Scorpionfish (15/13.5%)	14.6%	10%	10%	9.6%	13.6%	8.6%	8.4%	8%
Spiny lobster (15/13.5%)	12.6%	10%	9.9%	9.9%	11.6%	9.3%	9.3%	9.5%
Squirrelfish snapper (15/13.5%)	2.1%	4.5%	4.7%	2.9%	2.2%	5%	5.3%	3.7%
Triggerfish (30/27%)	19%	27%	27.8%	27.5%	18%	25%	25.1%	24.8%
Octopus (5/4.5%)	7.5%	3.1%	3.4%	3.3%	6%	2.6%	2.7%	2.4%
Swimming crab**	5.2%	4.8%	5.4%	4.9%	5%	4.4%	4.9%	4.5%
Lizard/snake fish**	3.0%	2.1%	2.2%	2.2%	3%	2.2%	2.0%	2.1%
Moray**	2.4%	2.3%	2.0%	2.5%	2.9%	3%	3.1%	3.8%
Duckbill**	2.2%	3.7%	3.7%	4.9%	2.1%	3.2%	3.0%	4.3%
Toby**	1.6%	1.3%	1.4%	1.0%	1.7%	1.5%	1.7%	1.6%
Others**	12.2%	12.9%	11.3%	12.9%	17.6%	18.0%	17.6%	18.2%
% False Positive	26.6%	27.1%	26.0%	28.4%	32.3% (22.3%)	32.3% (22.3%)	32.3% (22.3%)	34.5% (24.5%)

**Species that were not included in the pseudo diet species mix. Only the unspecified species representing more than 1.5% of the modeled diet are listed in the table. The rest are grouped as a single 'others' category.

Box highlights the best results (i.e., the lowest proportion of false positives) for the no noise simulation.

Removing False Positives

From the 8 simulations run for each of the above two pseudo diet compositions, the diet/FA set pairing which yielded the best simulation results (i.e., the lowest proportion of false positives) were selected. We used only the trials run with no noise, as the 10% misclassifications specified for those runs might confuse the issue. For each of these selected 'best' trials (highlighted in boxes in Tables 14, 15), the false positive groups that represented more that 0.01% of the modeled diet were removed. The pseudo diets were then re-modeled for each trial to examine whether the modeled diets would reflect the pseudo diets more accurately, without species presenting a potential overlap in their FA signatures. Results are presented in Tables 16 and 17.

The best of Trial 1.2

Two simulations yielded the lowest proportion of false positives in the pseudo diet composition of Trial 1 (Table 14) when run with no noise: the diet modeled on the modified 1 and 2 FA sets with no noise. The following false positive groups were removed prior to conducting the next set of simulations: scorpionfish, toby, duckbill, flower snapper, lizard/snakefish.

Table 16.--Simulations results for Trial 1.2 with major false positives removed. Also listed are the new false positive groups (**) and the total proportion of new false positives as a measure of the simulation success.

	No Noise	
	Modified 1	Modified 2
Spiny lobster (15%)	13.6% *(91% correct)*	12.8% *(85% correct)*
Pink snapper (15%)	8.8% *(59% correct)*	8.9% *(59% correct)*
Moray eel (35%)	38.8% *(4% overestimated)*	37.8% *(4% overestimated)*
Wrasse (35%)	25.9% *(74% correct)*	27.2% *(78% correct)*
Octopus**	1.2%	1.5%
Boarfish**	1.1%	0.8%
Bigeye**	1.0%	0.9%
Others**	9.8%	10.1%
% false positives	13.1%	13.3%

**Species that were not included in the pseudo diet species mix

Boxplots of each run follow.

Figure 33.--Boxplot of the QFASA simulation results (1000 iterations) for diet Trial 1.2, using the modified 1 FA set and no noise, on all species groups except with scorpionfish, toby, duckbill, flower snapper, and lizard/snakefish removed. The diet composition specified is represented in plots as "a".

Figure 34.--Boxplot of the QFASA simulation results (1000 iterations) for diet Trial 1.2, using the modified 1 FA set and no noise, on all species groups except with scorpionfish, toby, duckbill, flower snapper, and lizard/snakefish removed. The diet composition specified is represented in plots as "a".

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The best of Trial 3.2

The lowest proportion of false positives using no noise resulted from modeling the diet composition of Trial 3 on the modified 1 FA set. The following six false positive groups were removed prior to conducting another simulation: swimming crab, lizard/snakefish, moray eels, duckbill, toby, and wrasse/hogfish/coris.

Table 17.--Simulation results for Trial 3.2 with major false positives removed. Also listed are the new false positive groups (**) and the total proportion of new false positives as a measure of the simulation success.

	No Noise
Parrotfish (20%)	17.8% (89% correct)
Scorpionfish (15%)	15.6% (100% correct)
Spiny lobster (15%)	12.8% (85% correct)
Squirrelfish snapper (15%)	8.0% (53% correct)
Triggerfish (30%)	28.9% (96% correct)
Octopus (5%)	3.8% (76% correct)
Box crab**	2.0%
Cutthroat/snake eel**	1.2%
Butterfly/forcep/pennantfish **	1.1%
Bigeye**	1.1%
Others**	7.7%
% False Positive	13.1%

**Species that were not included in the pseudo diet species mix
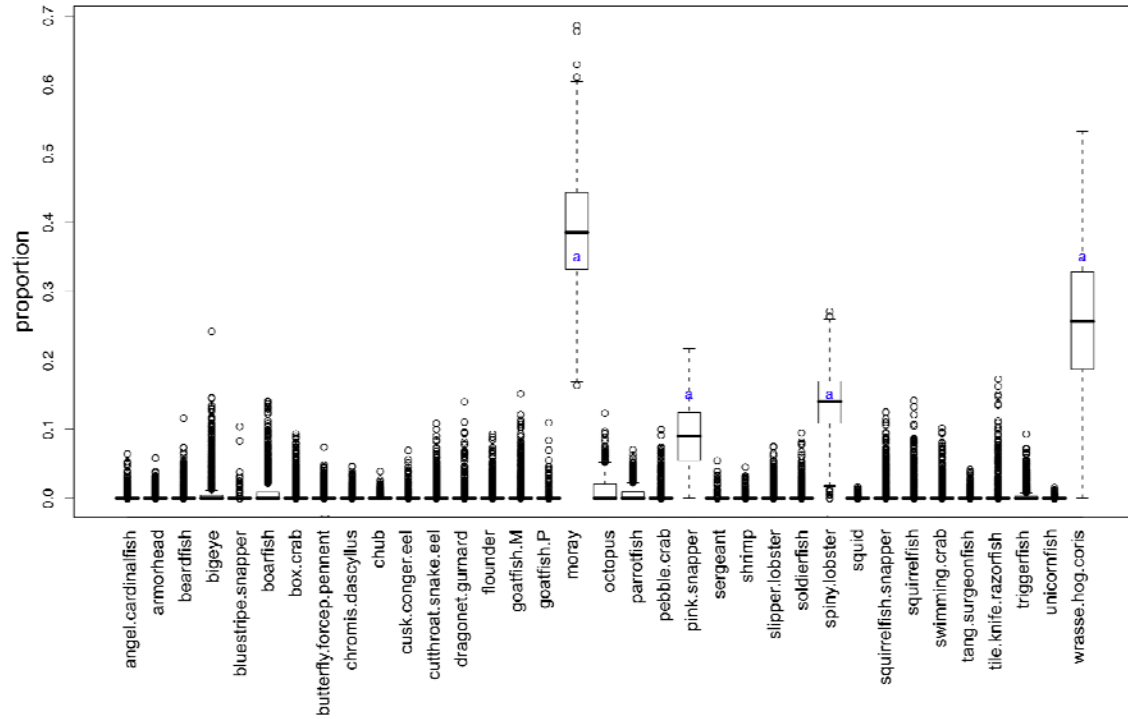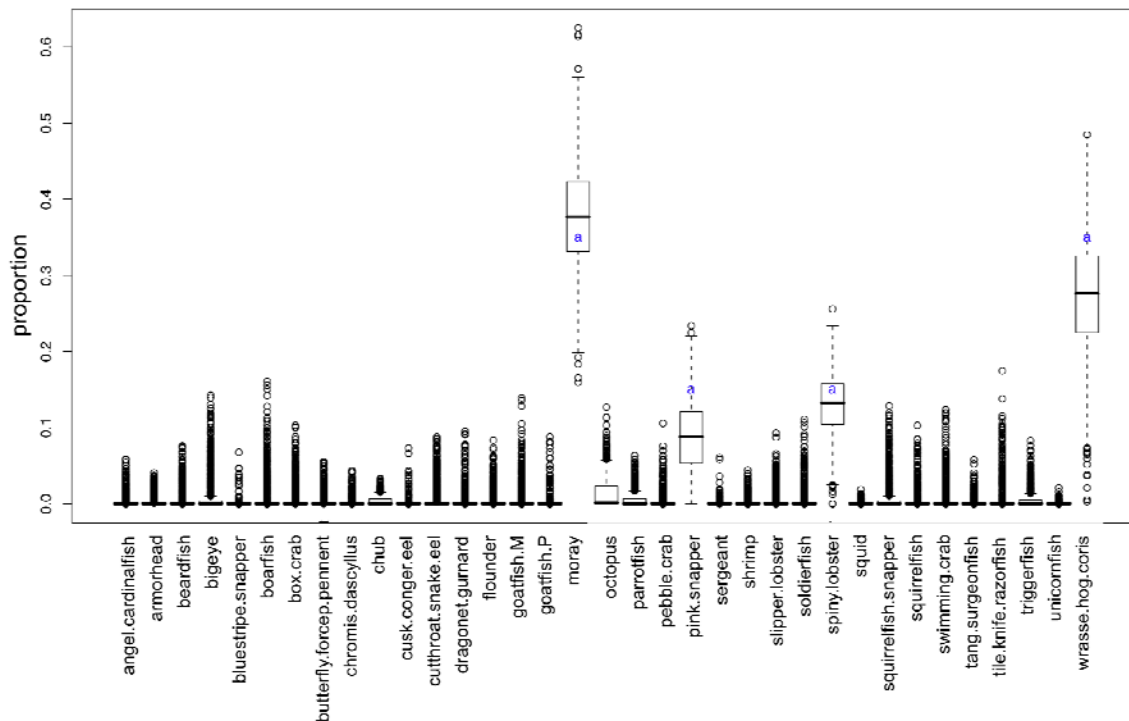
The boxplot of this run follows.
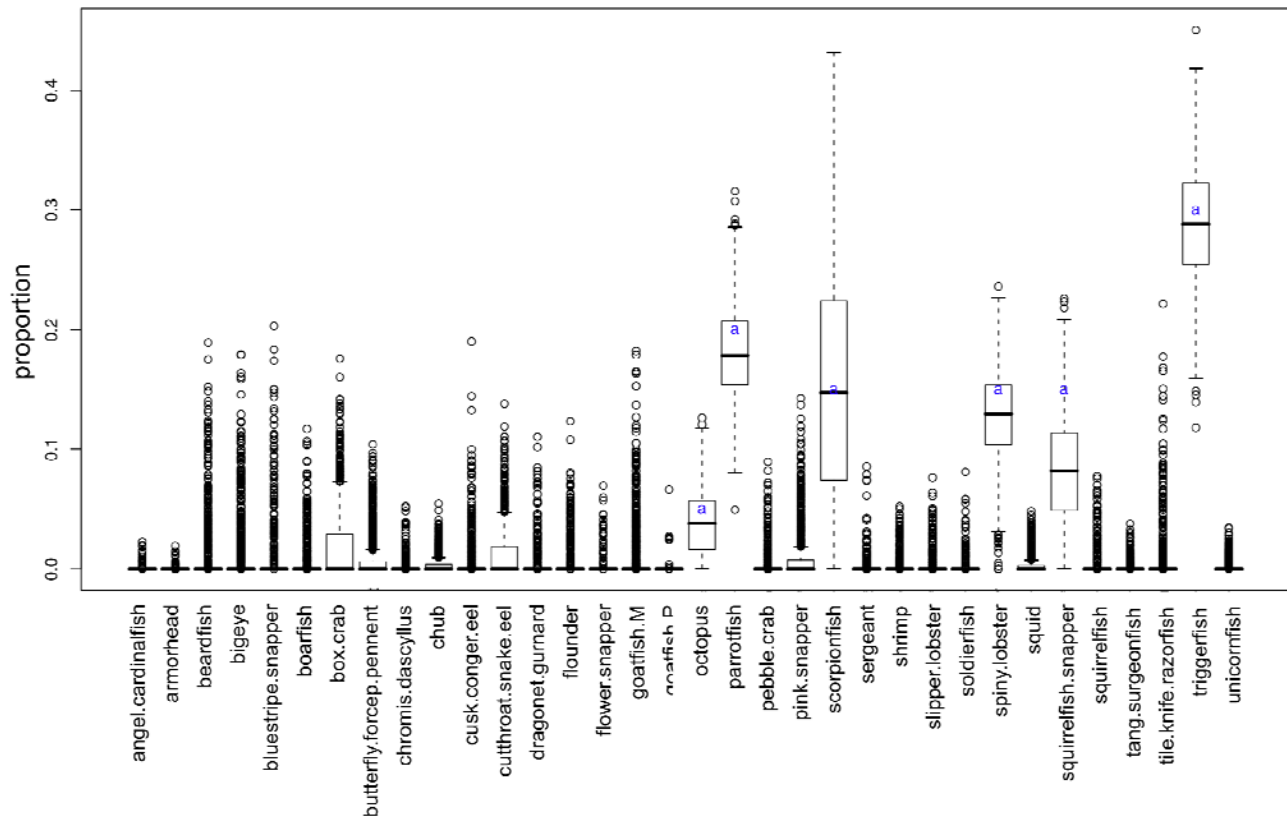
Figure 35.--Boxplot of the QFASA simulation results (1000 iterations) for diet Trial 3.2, using the modified 1 FA set and no noise, on all species groups except with swimming crab, lizard/snakefish, moray eels, duckbill, toby, and wrasse/hogfish/coris removed. The diet composition specified is represented in plots as "a".

Simulating 10 Species of Interest

In addition to the simulations performed above to explore the identification and estimation of prey species in simulations of pseudo diets, we were asked to perform a final simulation, which included a large number of species (10), all at the same specified level in diet. Previous QFASA simulations (including using other prey databases) have shown that pseudo diets containing a large number of species are generally not well-simulated for a reason that has to do with the mathematical procedures used. At this stage, until further work is developed, we do not yet fully understand what the mathematical issues are, but simulations may be effected by the splitting process, by sample sizes of prey species or groupings (e.g., when vastly differing sample sizes of prey exist, or when prey specified in the simulations are represented by < 30-40 individuals, greater errors appear across all prey and simulations), and by the number and levels of prey specified in the pseudo diet, that may have little to do with how well prey are actually differentiated or estimated in the diets of real predators (S. Iverson and W. Blanchard, unpublished data). Thus, when erroneous results appear in such simulations, we do not yet know whether such results would occur in modeling the actual predator or whether they merely reflect something in the simulation process itself. Nevertheless, we performed these simulations to investigate the success of estimates.

10 Species Mix:

1. Armorhead: 10% ($n = 20$)
2. Moray: 10% ($n = 135$)
3. Spiny lobster: 10% ($n = 60$)
4. Octopus: 10% ($n = 40$)
5. Squirrelfish.snapper: 10% ($n = 21$)
6. Flower.snapper: 10% ($n = 19$)
7. Squid: 10% ($n = 15$)
8. Tang-surgeonfish: 10% ($n = 154$)
9. Triggerfish: 10% ($n = 39$)
10. Flounder: 10% ($n = 117$)

All simulations were modeled on the modified 1 and 2 FA sets, given that previous results indicated that modeling on these sets yielded the best results. The simulations were first performed using 10% of each of the 10 specified prey groups. Then modeling was re-performed, after sequentially removing false positives. These results are presented in Table 18.

Table 18.--Results of QFASA simulations for a diet composed of the 10 species mix above (10% each), using the modified 1 and 2 FA sets. Results represent the proportion (%) each species appearing in the modeled diet. The false positive value is the total proportion of misclassified species. Species specified in the diet are highlighted in blue. In Trial 1, all species were used in modeling. In Trial 2, the following false positive species were excluded from: cutthroat/snake eel, shrimp and toby. In Trial 3, one additional false positive, beardfish, was removed.

	Trial 1 (n = 40)		Trial 2 (n = 37)		Trial 3 (n = 36)	
	Modified 1 set (%)	Modified 2 set (%)	Modified 1 set (%)	Modified 2 set (%)	Modified 1 set (%)	Modified 2 set (%)
angel_cardinalfish	1.9	1.9	1.9	2.1	1.9	1.9
Armorhead	**8.0**	**7.7**	**9.8**	**9.7**	**9.8**	**10.3**
Beardfish	5.8	3.8	6.6	5.3	---------------	-------------
Bigeye	0.5	0.4	0.5	0.4	0.3	0.3
bluestripe.snapper	1.1	1.6	0.9	1.1	0.8	0.7
Boarfish	1.5	1.6	1.5	1.6	0.6	0.7
box.crab	0.6	1.1	1.5	1.7	2.9	2.6
butterfly.forcep.pennant	2.1	1.2	2.2	1.3	1.8	1.1
chromis.dascyllus	0.8	1.0	0.6	0.7	0.9	0.9
Chub	0.4	0.4	0.3	0.2	0.3	0.2
cusk_conger_eel	0.5	0.6	1.2	1.1	0.3	0.3
cutthroat.snake.eel	3.5	4.3	---------------	---------------	---------------	-------------
dragonet.gurnard	0.7	0.7	1.0	1.0	0.3	0.3
Duckbill	1.8	2.5	5.1	5.2	4.9	5.4
Flounder	**5.3**	**3.9**	**3.8**	**3.2**	**2.4**	**3.0**
flower.snapper	**1.2**	**1.8**	**1.8**	**2.8**	**3.8**	**3.3**
goatfish.M	0.8	0.9	1.3	1.6	0.1	0.2
goatfish.P	0.0	0.0	0.0	0.0	0	0.0
lizard.snakefish	2.2	1.4	1.9	1.2	0.9	0.7
Moray	**6.6**	**7.0**	**6.9**	**6.9**	**7.3**	**7.8**
Octopus	**8.1**	**8.1**	**7.4**	**7.5**	**9.3**	**9.2**
Parrotfish	0.9	0.9	0.6	0.5	0.9	1.1
pebble.crab	1.0	0.6	1.5	1.0	1.6	1.3
pink.snapper	0.7	0.4	0.7	0.5	0.4	0.5
Scorpionfish	1.6	1.7	1.5	1.7	1.2	1.7
Sergeant	0.5	0.5	0.3	0.4	0.2	0.2
Shrimp	3.8	3.8	---------------	---------------	--------------	-------------
slipper.lobster	2.6	3.6	3.7	4.6	0.9	0.9
Soldierfish	1.3	2.4	1.0	1.4	0.8	1.1
spiny.lobster	**1.5**	**1.3**	**1.6**	**1.5**	**4.6**	**4.9**
Squid	**9.4**	**9.1**	**10.4**	**10.0**	**9.7**	**9.4**
squirrelfish.snapper	**5.2**	**6.3**	**5.1**	**6.4**	**10.4**	**9.6**
Squirrelfish	0.9	1.6	1.0	1.9	1	1.6
swimming.crab	0.6	0.7	0.4	0.4	1.1	1.0
tang.surgeonfish	**5.1**	**6.0**	**5.6**	**6.2**	**6.5**	**6.9**
tile.knife.razorfish	1.5	1.4	1.8	1.7	0.9	0.9
Toby	2.6	3.1	---------------	---------------	--------------	-------------
Triggerfish	**4.0**	**3.3**	**5.7**	**5.6**	**5.9**	**6.2**
Unicornfish	1.1	0.8	0.9	0.7	0.6	0.6
wrasse.hog.coris	2.2	0.6	1.8	0.9	2.8	1.8
% false positives	**45.5**	**45.5**	**41.7**	**40.2**	**30.5**	**29.4**

Simulations revealed that the removal of cutthroat/snake eels, shrimp and toby had minor impacts on the simulation results, with ~ 40% false positives remaining in simulated diets (Table 18, Trial 2). However, the removal of the beardfishes improved the modeling results of several species; the armorhead, the octopus, the squid, and the squirrelfish snapper were all successfully modeled upon the exclusion of beardfishes from the modeling process (Table 18, Trial 3). This may suggest an overlap in the FA signature of squirrelfish snapper and beardfish. Nevertheless, some species such as the flounder and the flower snapper were still underrepresented in the simulation results, and other false positive species, such as the duckbill, appeared. Problems arising from some species groups having large sample sizes and at least four having $n \le 21$ may have contributed to performance of the simulations, especially with a large number of species.

To investigate whether the underestimates of specified prey and appearance of false positives were a real function of the species FA signatures or contribution of mathematical issues in the current simulation process with a large number of species, the 10 prey groups were split into two sets of five prey groups each and remodelled to elucidate whether there may be numerical problems with simulating on 10 species, as has been found previously.

SET 1:   SET 2:

Armorhead	20%	Spiny lobster	20%
Flounder	20%	Squid	20%
Flower snapper	20%	Squirrelfish snapper	20%
Moray eel	20%	Tang/surgeonfish	20%
Octopus	20%	Triggerfish	20%

Two sets of simulations were performed. After each full simulation (Trial 1), the major false positives were sequentially removed (denoted by ---------), and the model was rerun (Tables 19 and 20, Trials 2-3 and 2-6, respectively). Figure 36 illustrates the boxplots of the simulation (Table 18, Trial 1) run with all 10 species at 10% and in comparison to one of the simulations (Table 19, Trial 1, SET 1) run with 5 of the 10 species at 20%.

Table 19.--Results of QFASA simulations obtained from modeling the diet of SET 1 above (20% each), using only the modified 1 FA set. Results represent the proportion (%) each species appeared in the diet after modeling. The false positive value is the total proportion of mis-classified species. Species specified in the diet are highlighted in blue. In trial 1, all species were used in modeling. In subsequent trials major false positives were sequentially removed (-----).

Species excluded	Trial 1 (*n*=40) None	Trial 2 (*n*=38) pink and squirrelfish snappers	Trial 3 (*n*=37) pink and squirrelfish snappers, lizard/snakefish	Trial 4 (*n*=36) pink and squirrelfish snappers, lizard/snakefish, beardfish
angel_cardinalfish	0.1	0.1	0.1	0.1
armorhead	**18.7**	**18.7**	**18.0**	**18.5**
beardfish	2.6	3.2	3.5	----------------------
bigeye	0.2	0.3	0.8	1.1
bluestripe.snapper	0.7	1.1	0.6	0.9
boarfish	0.7	0.9	1.6	1.1
box.crab	1.2	1.0	1.0	0.7
butterfly.forcep.pennant	0.3	0.3	0.2	0.2
chromis.dascyllus	0.0	0.0	0.0	0.0
chub	0.2	0.2	0.2	0.1
cusk_conger_eel	0.9	1.0	0.9	1.1
cutthroat.snake.eel	0.7	0.8	0.7	1.4
dragonet.gurnard	0.7	0.7	0.8	0.6
duckbill	0.5	1.4	1.6	2.2
flounder	**12.1**	**12.7**	**13.5**	**14.1**
flower.snapper	**11.1**	**12.9**	**14.4**	**15.3**
goatfish.M	0.0	0.0	0.0	0.0
goatfish.P	0.0	0.0	0.0	0.0
lizard.snakefish	2.4	3.4	----------------------	----------------------
moray	**17.8**	**18.6**	**19.4**	**18.9**
octopus	**15.9**	**15.5**	**16.1**	**16.3**
parrotfish	0.0	0.0	0.1	0.0
pebble.crab	0.2	0.1	0.1	0.1
pink.snapper	2.4	----------------------	----------------------	----------------------
scorpionfish	1.5	1.4	2.0	2.2
sergeant	0.0	0.0	0.0	0.0
shrimp	0.3	0.2	0.2	0.2
slipper.lobster	0.1	0.1	0.0	0.0
soldierfish	0.4	0.4	0.3	0.4
spiny.lobster	0.2	0.3	0.3	0.2
squid	1.3	1.6	1.2	1.9
squirrelfish.snapper	3.4	----------------------	----------------------	----------------------
squirrellfish	1.5	1.3	1.0	1.0
swimming.crab	0.0	0.0	0.0	0.0
tang.surgeonfish	0.2	0.1	0.1	0.1
tile.knife.razorfish	0.6	0.3	0.2	0.3
toby	0.7	0.7	0.8	0.7
triggerfish	0.1	0.1	0.0	0.0
unicornfish	0.1	0.2	0.2	0.1
wrasse.hog.coris	0.1	0.1	0.0	0.0
% false positives	**24.3**	**21.3**	**18.5**	**16.7**

Table 20.—Results of QFASA simulations obtained from modeling the diet of SET 2 above (20% each), using only the modified 1 FA set. Results represent the proportion (%) each species appeared in the diet after modeling. The false positive value is the total proportion of misclassified species. Species specified in the diet are highlighted in blue. In Trial 1, all species were use in modeling. In subsequent trials, major false positive were sequentially removed (-----).

	Trial 1 (n = 40)	Trial 2 (n = 39)	Trial 3 (n = 38)	Trial 4 (n = 37)	Trial 5 (n = 36)	Trial 6 (n = 35)
Species excluded	None	duckbill	duckbill, swimming crab	duckbill, swimming crab, parrotfish	duckbill, swim. crab, parrot-fish, cutthroat/ snake eel	duckbill, swim. crab, parrotfish, cutthroat/ snake eel, beardfish
angel_cardinalfish	2.0	1.4	1.6	1.3	1.4	1.2
armorhead	0.0	0.0	0.0	0.0	0.0	0.2
beardfish	2.9	2.8	2.2	2.5	3.5	--------------
bigeye	0.1	0.1	0.2	0.2	0.4	0.5
bluestripe.snapper	0.1	0.1	0.1	0.1	0.1	0.1
boarfish	0.5	1.1	1.0	1.0	0.8	0.5
box.crab	0.5	0.6	1.5	2.2	1.6	1.5
butterfly.forcep.pennant	1.2	1.2	1.1	0.7	0.7	1.0
chromis.dascyllus	1.7	1.8	1.2	1.9	1.5	1.9
chub	0.4	0.2	0.3	0.5	0.7	0.4
cusk_conger_eel	0.2	0.1	0.2	0.2	0.3	0.3
cutthroat.snake.eel	2.9	4.5	4.6	4.1	--------------	--------------
dragonet.gurnard	0.2	0.1	0.1	0.2	0.1	0.1
duckbill	4.8	----------	---------------	---------------	--------------	--------------
flounder	0.7	1.1	1.2	1.2	0.7	0.6
flower.snapper	0.0	0.1	0.0	0.0	0.1	0.3
goatfish.M	0.3	0.2	0.3	0.2	0.1	0.2
goatfish.P	0.0	0.0	0.0	0.0	0.0	0.0
lizard.snakefish	0.5	0.5	0.5	0.8	0.7	0.7
moray	0.6	0.8	0.6	0.6	0.4	0.7
octopus	1.1	1.2	1.4	1.7	1.1	1.1
parrotfish	3.0	3.2	3.4	--------------	--------------	--------------
pebble.crab	1.7	1.9	1.6	1.7	1.3	2.0
pink.snapper	0.1	0.2	0.2	0.1	0.0	0.1
scorpionfish	0.4	0.6	1.0	0.6	0.8	0.9
sergeant	0.3	0.2	0.2	1.8	1.7	1.5
shrimp	1.4	1.4	1.5	0.9	1.7	2.0
slipper.lobster	0.3	0.3	0.4	0.2	0.2	0.2
soldierfish	0.6	0.5	0.5	0.4	0.2	0.3
spiny.lobster	**10.3**	**9.8**	**12.0**	**12.4**	**14.1**	**14.4**
squid	**19.6**	**19.7**	**19.8**	**19.5**	**19.6**	**19.5**
squirrelfish.snapper	**3.5**	**5.9**	**6.5**	**6.7**	**10.2**	**12.9**
squirrellfish	0.1	0.1	0.0	0.1	0.0	0.1
swimming.crab	3.9	3.9	---------------	---------------	--------------	--------------
tang.surgeonfish	**13.0**	**13.1**	**13.1**	**14.1**	**14.3**	**14.3**
tile.knife.razorfish	1.0	0.7	0.7	0.5	0.6	0.5
toby	1.2	1.8	1.0	0.8	0.6	0.6
triggerfish	**16.1**	**16.2**	**16.9**	**18.0**	**18.0**	**16.8**
unicornfish	0.5	0.4	0.4	0.6	0.8	0.8
wrasse.hog.coris	2.1	2.3	2.6	2.0	1.7	1.7
False Positive	**37.3**	**35.4**	**31.6**	**29.1**	**23.8**	**22.0**

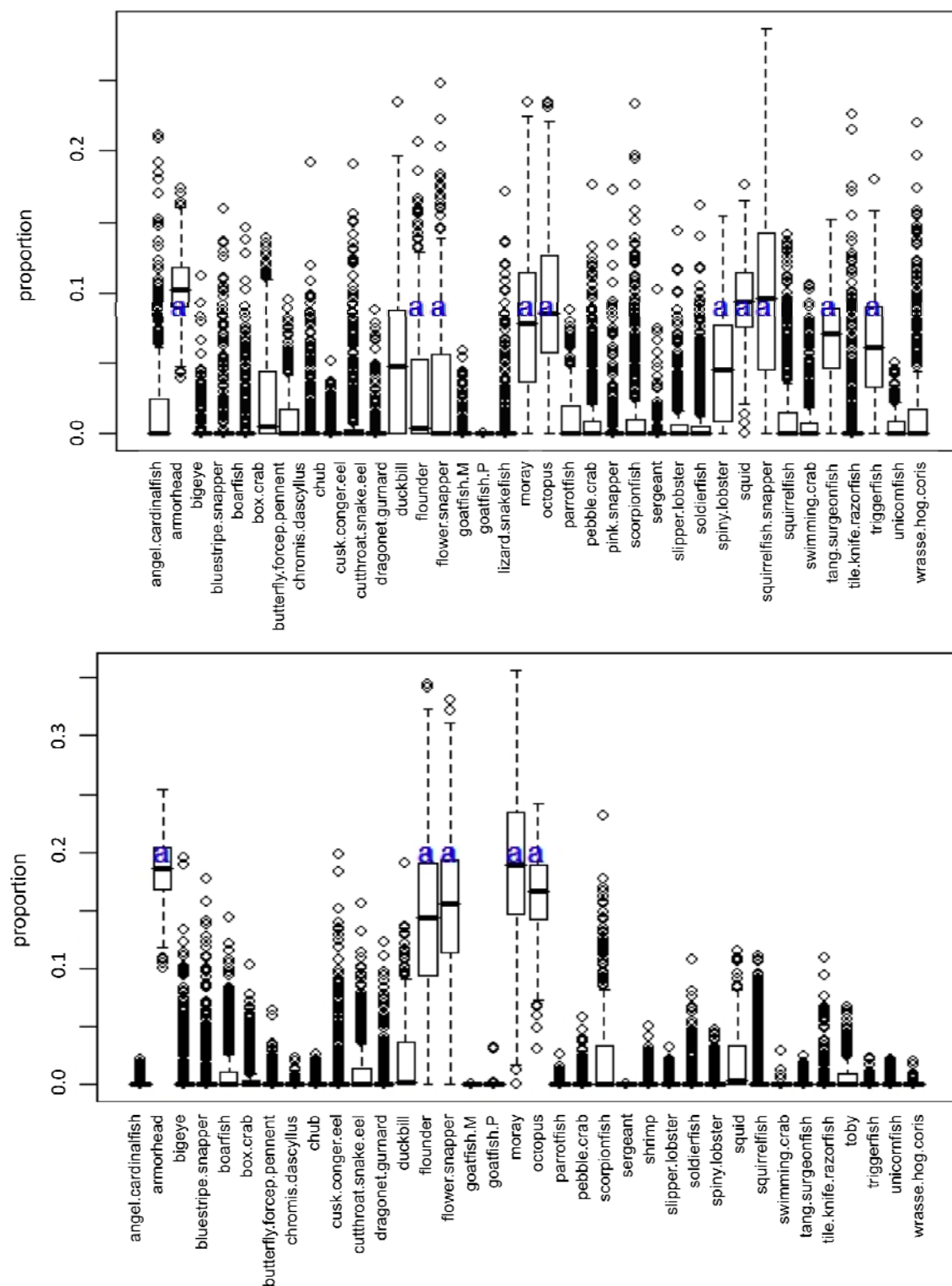Figure 36. Boxplot contrasting the QFASA simulation results for a diet of upper: 10% armorhead, 10% flounder, 10% flower snapper, 10% moray, 10% octopus, 10% spiny lobster, 10% squid, 10% squirrelfish snapper, 10% tang/surgeonfish, and 10% triggerfish, and lower: 20% armorhead, 20% flounder, 20% flower snapper, 20% moray, and 20% octopus (trial 1, Tables 18 and 19). The blue 'a' is a visual marker of the proportion of each prey.

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Summary of Prey Simulations

The results of previous QFASA simulations have suggested that some numerical problems arise when including a large number of species in simulations or when large differences in sample sizes between the specified species exist. Given that when simulations were run with five instead of ten of the species specified, 1) all species specified in the diet were better estimated, and 2) the number of total false positives were lower; the current simulation results appear to corroborate this observation. The simulations conducted with the two species sets suggested that the 10 species of interest can be characterized on the basis of their FA signature, although some overlap was clearly detected. The removal of certain species and remodeling process suggests a way to improve our understanding of these overlaps. Nevertheless, out of all 40 prey groups, the species specified were those that appeared most significantly in the simulated diets.

In general, one can perhaps best see the results of simulations most easily when examining the graphical illustrations of simulations, including those originally performed, those using modified FA sets, and those with removal of certain prey. These show, that in terms of distinguishing prey groupings from all other 40 prey groupings in the diet, in most cases the major prey species in the specified diets are being estimated and many times estimated at their absolute levels fairly well. This is encouraging, given the huge complexity of this prey database and ecosystem. If, in the modeling of actual monk seal diets, the model performs as well or better than in simulations, then it would mean that QFASA is, overall, detecting the major prey that monk seals are utilizing. However, one has to be very aware, in this ecosystem – which is pushing the very limits of QFASA, of several important issues: 1) that of overlap in some problematic species, as well as 2) noise in terms of false positives. The second issue may be easier to deal with. As illustrated by the simulation graphs in general, species that appear in actual diet estimates at very low levels may have to be taken with a grain of salt; that is, while minor occurrences in diets could be real, such levels may also simply reflect noise. However, if such prey are indeed very minor in monk seal diets in any case, then they likely would not matter significantly to the monk seal population in general. The more significant issue to be aware of, is which species may be exhibiting most overlap with one another such that it results in the appearance of significant false positives and underestimation of actual prey of importance - and to then determine how best to deal with those prey in terms of interpreting modeling results for actual monk seal diet estimates. We thus performed one additional analysis to address these issues in a somewhat different manner than simulating the diets of pseudo seals.

Prey Modeled on Other Prey

The simulations of pseudo seals provide powerful insight into the success of the QFASA model in differentiating prey and in estimating proportions specified. However, to an extent that is not yet fully understood mathematically, simulations may be effected by the splitting process, by sample sizes of prey species or groupings, and by the number and levels of prey specified in the pseudo diet, that may have little to do with how well prey are actually differentiated or estimated in the diets of real predators (S. Iverson and W. Blanchard, unpublished data). Thus, we performed one additional set of modeling, which is closer to the way in which real predators are modeled. The aim of this modeling was to simply model each individual prey grouping on all

other prey in the database, including itself. This process of modeling the prey species as predator should provide the best test of the probability of correctly identifying a given prey and/or likelihood of mistaking it for other prey, as well as to directly identify which prey it is most-likely to be mistaken for. This process should also provide insight into how our groupings appear to be working in terms of both successfully combining FA signatures and thus being able to interpret model results ecologically.

The procedure undertaken was the following: for each of the 40 species groups (e.g., beginning with "angel.cardinalfish"), from the individuals sampled (i.e., $n = 38$ in that group, Table 2), the individuals were randomly split into two groups of equal sample size: a simulation group and a modeling group. An average of the simulation group was then modeled in QFASA on the modeling group and all other 39 prey species groups. This process of splitting and modeling was repeated 1000 times, as before. The results of this modeling series should indicate the likelihood of that selected prey being identified as itself or as other prey. We used two additional FA sets for these models. Given the relative success of the modified 1 and 2 extended FA sets, we used those sets with minor corrections specific to monk seals: the first new set was almost identical to the former two, but simply replaced two FAs that were either more or less reliably measured in the NWHI ecosystem and monk seals (as opposed to northern temperate), while the second set removed any FA that was measured at close to zero in captive or wild monk seals, regardless of whether it had a CC with little variability, to remove any influence of noise around zero having significant impacts on our estimates (Appendix C). Since this is the way we planned to model actual monk seals, these were deemed the best sets for this procedure.

The results of this modeling series were first summarized, as a whole, in image plots, which allow an overall view of modeling success and of reliably identifying a prey group in the QFASA model (Fig. 37). The degree to which a bright yellow square is matched with the same prey group on the X and Y axis indicates the success of estimating that prey group as itself out of all 39 other prey groups in the QFASA model. The relatively consistent diagonal line formed by the yellow squares (Fig. 37a and b) clearly demonstrates that each prey was generally strongly identified as itself in the QFASA modeling. However, an example of where a prey group was in part incorrectly identified is the plot for the angel.cardinalfish group in Fig. 37b.

a) MS FA Set
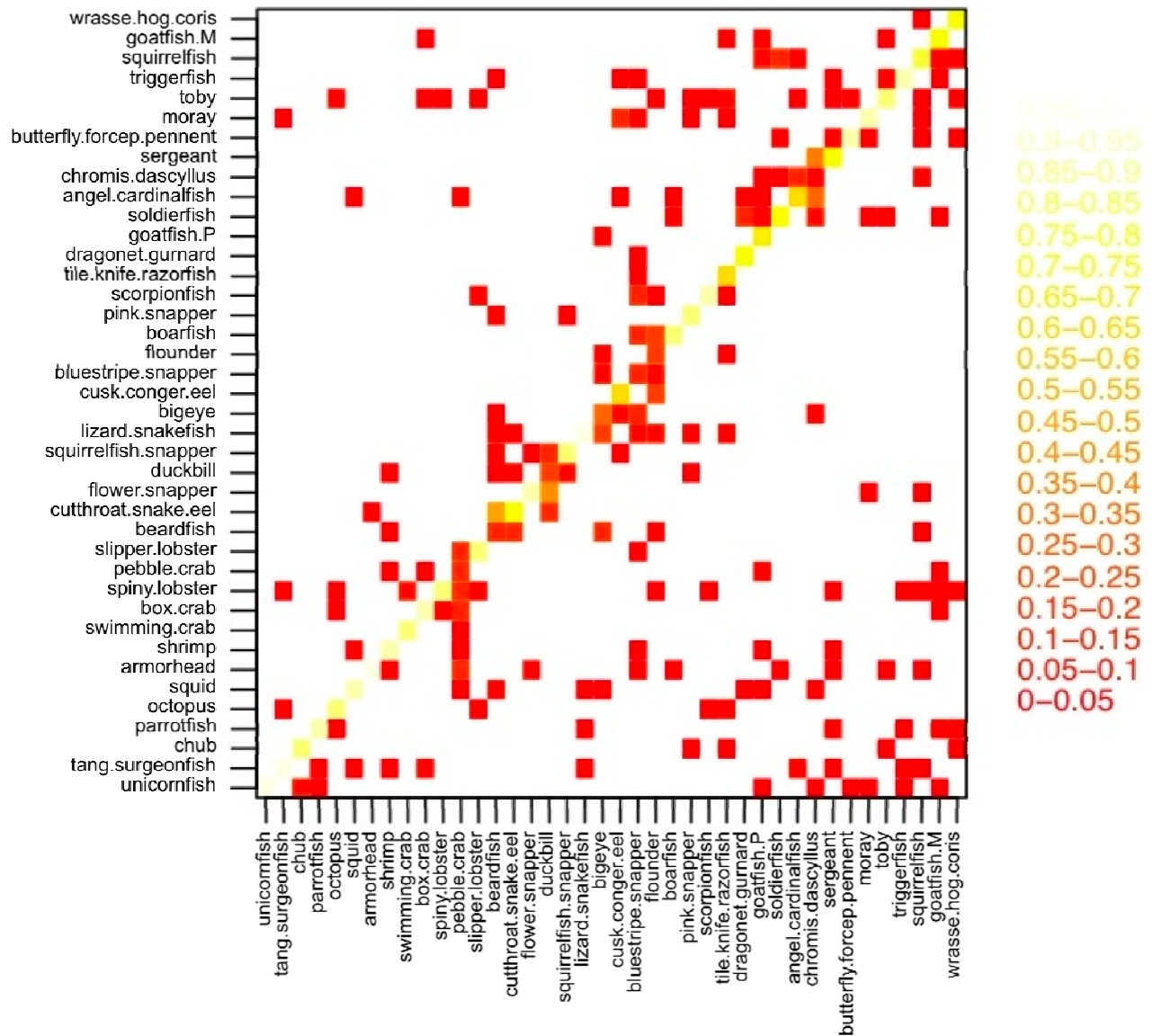
b) MS FA Minus Set
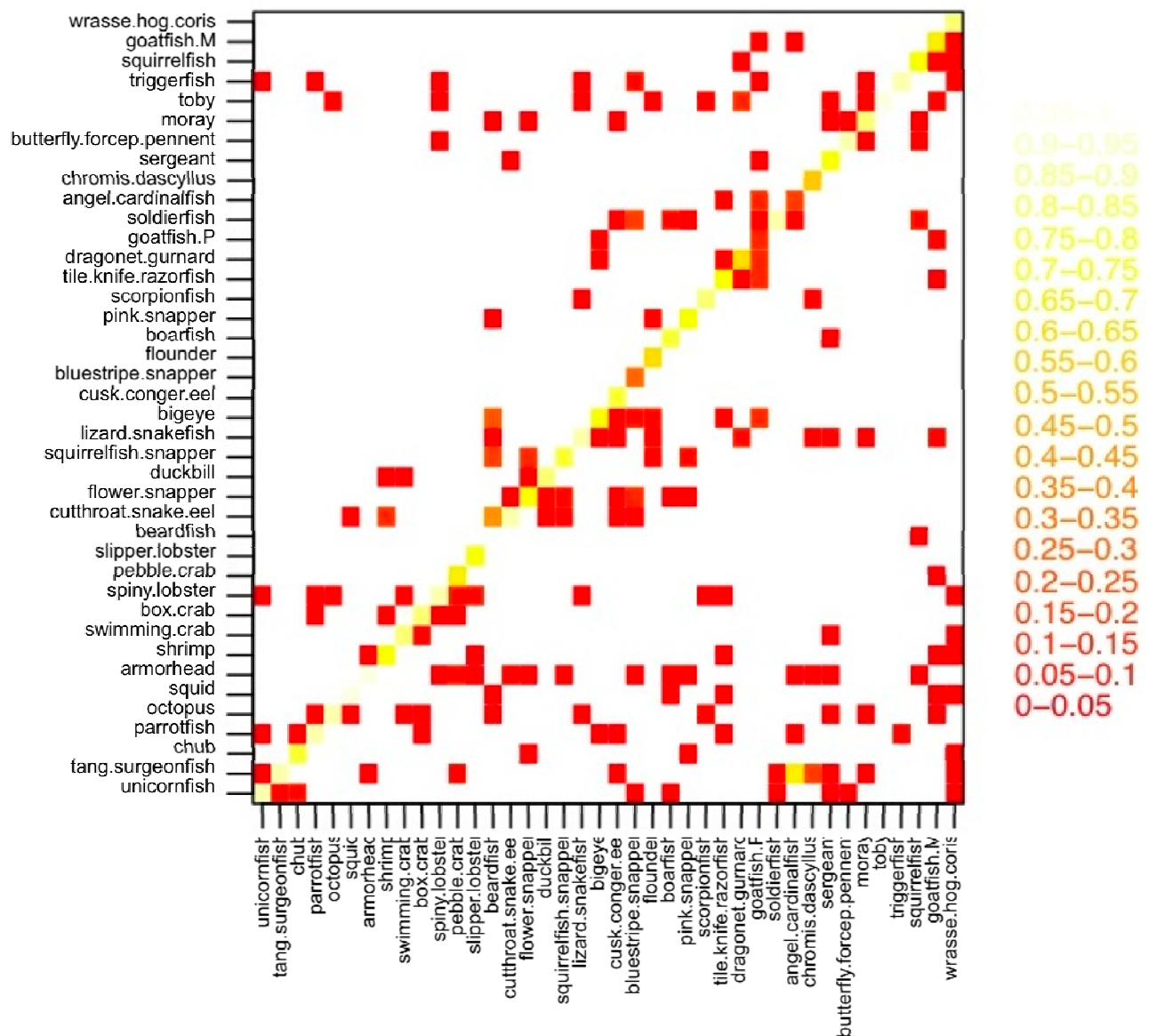
Figure 37.--Image plots depicting the degree of classifying (estimating) a prey group as itself out of all 40 prey groups using a) the MS FA set and b) the MS minus FA set in the QFASA model (Appendix C). Brightest yellow squares indicate that 95-100% of the prey group modeled (on the X axis) was estimated as a given prey on the Y axis. At the farthest extreme, deepest red squares indicate that 0-5% of the prey group modeled (on the X axis) was estimated as a given prey on the Y axis (i.e., representing outliers or noise). The diagonal line formed by most yellow squares indicates the success, overall, in estimating prey groups as themselves.

The following plots (Fig. 38) present the specific results for each of the 40 prey species groups (in alphabetical order).
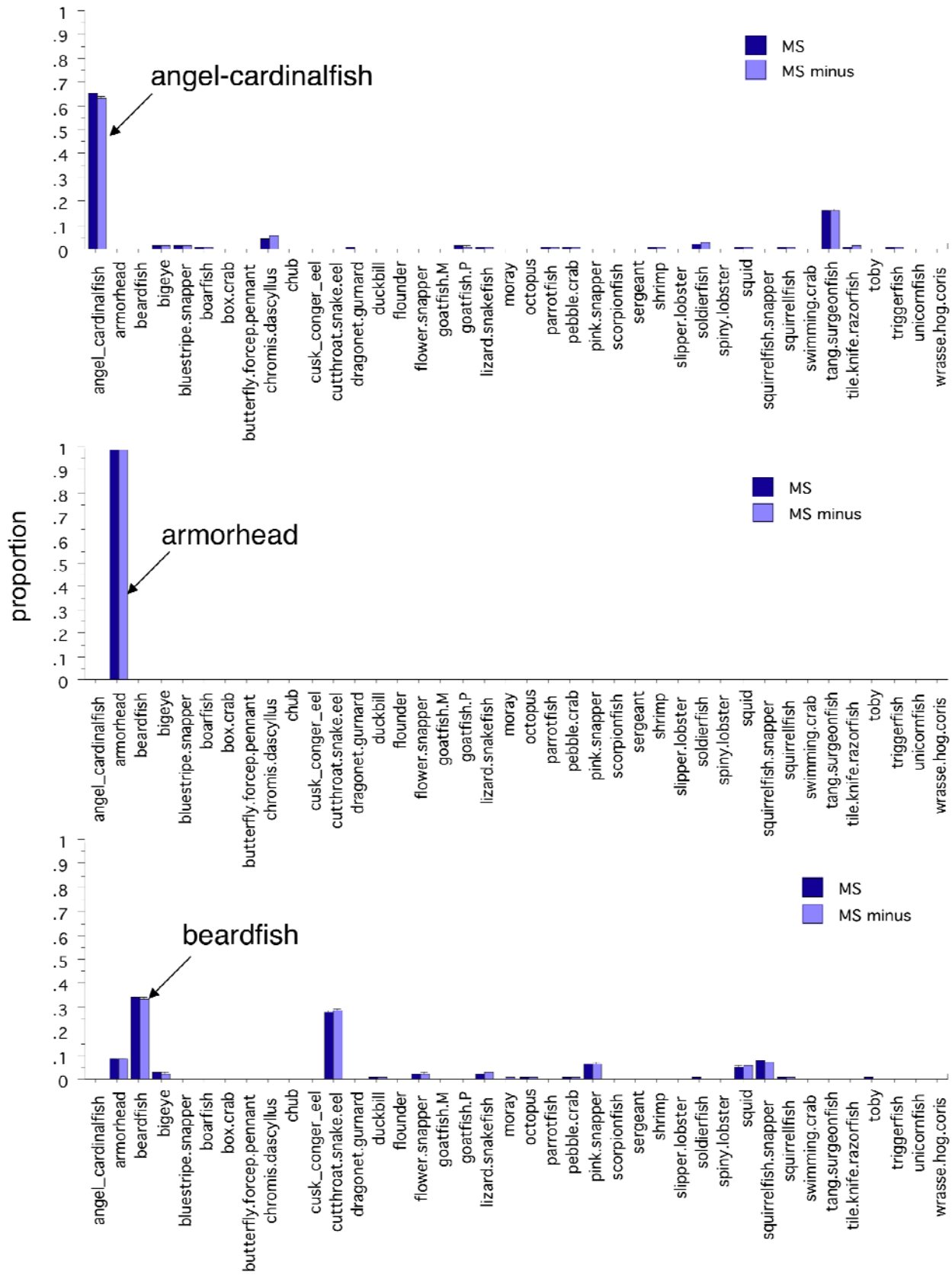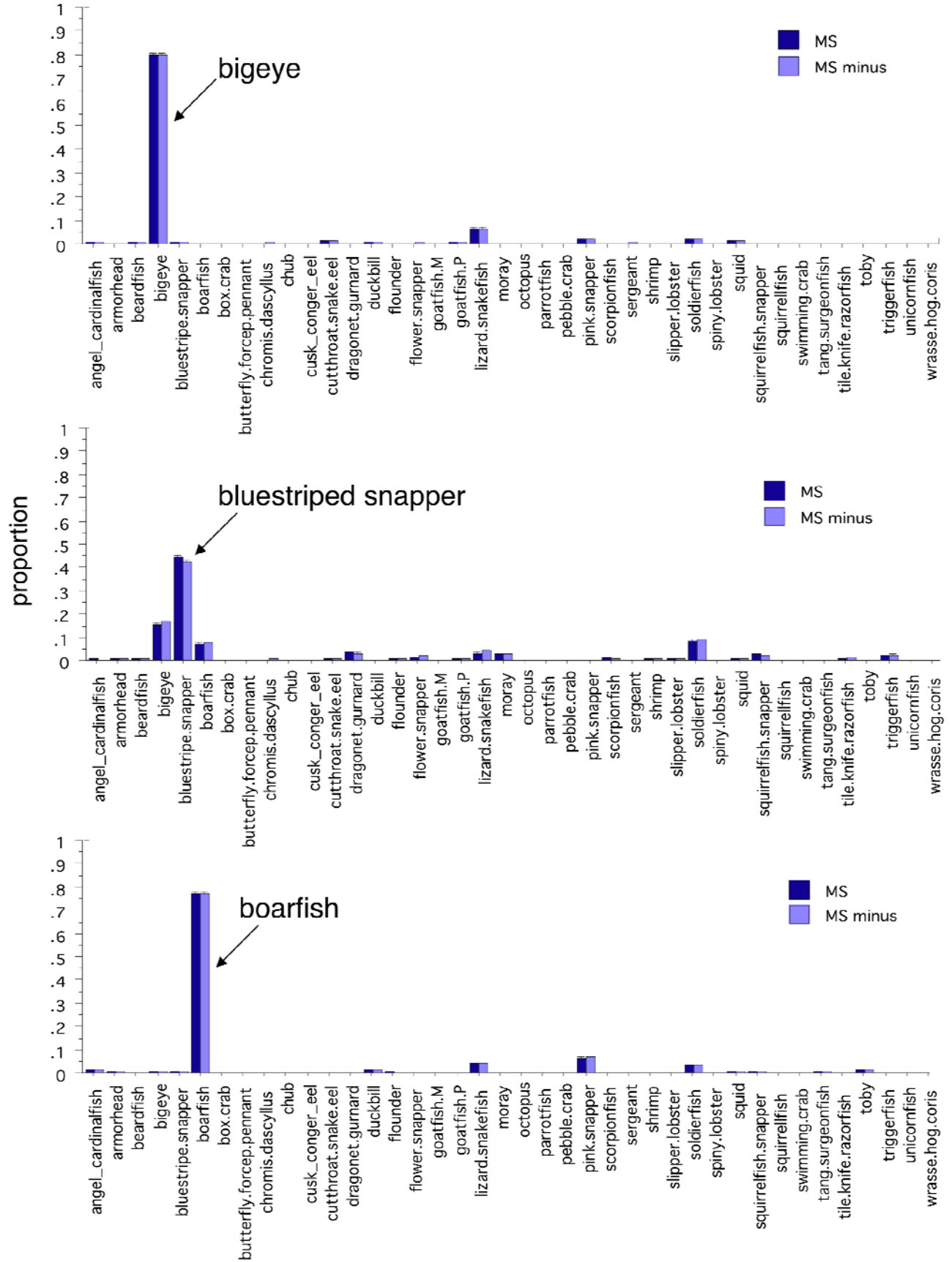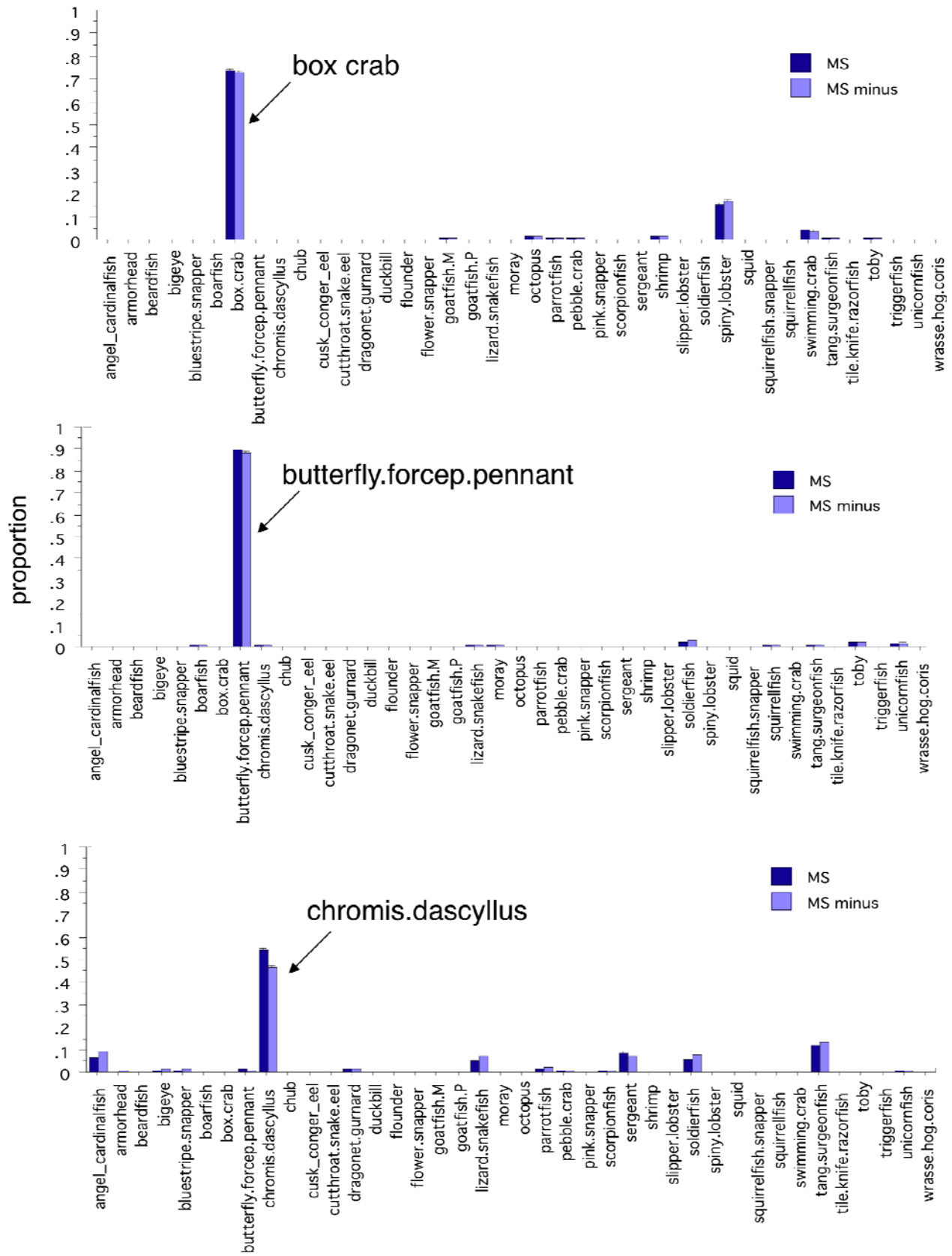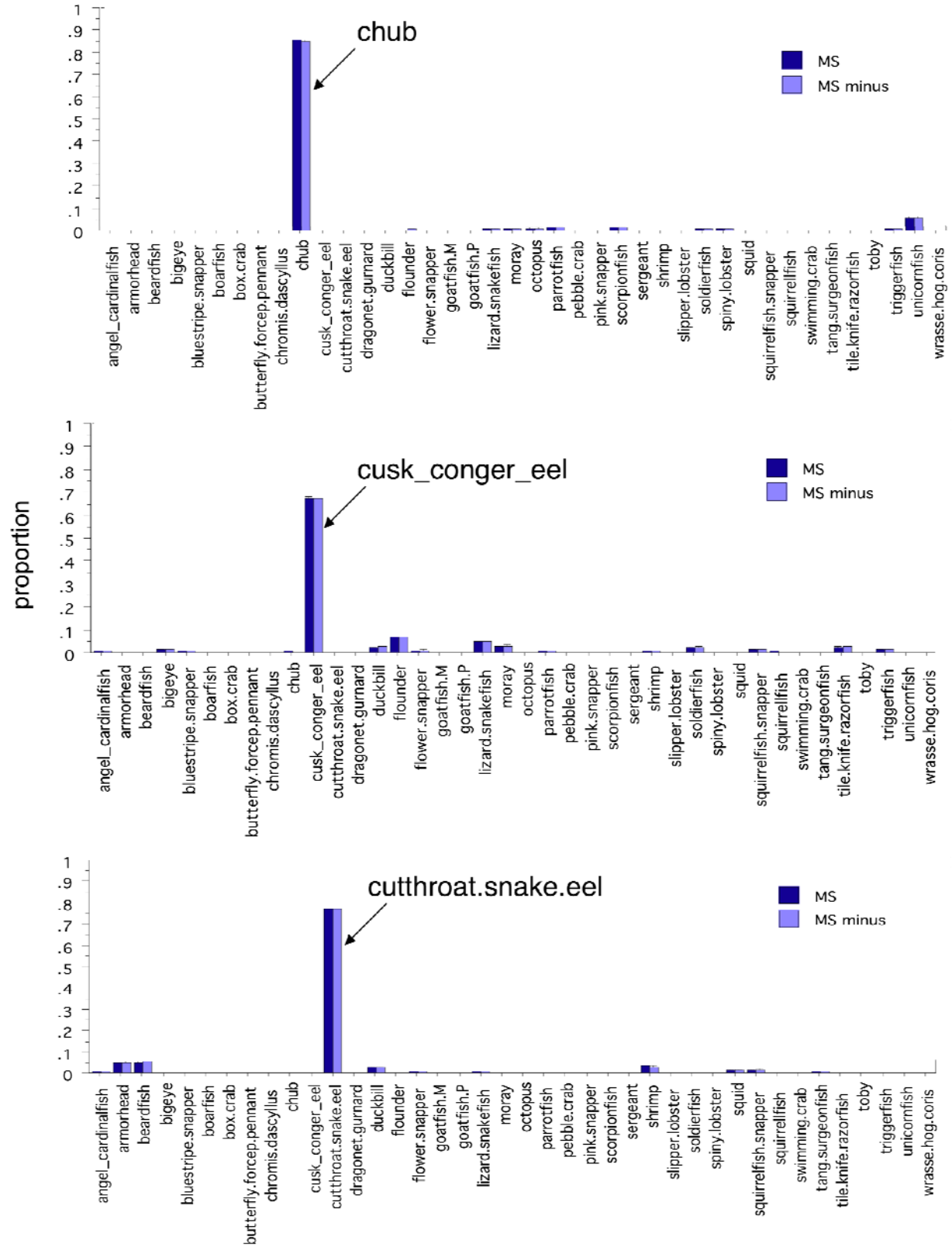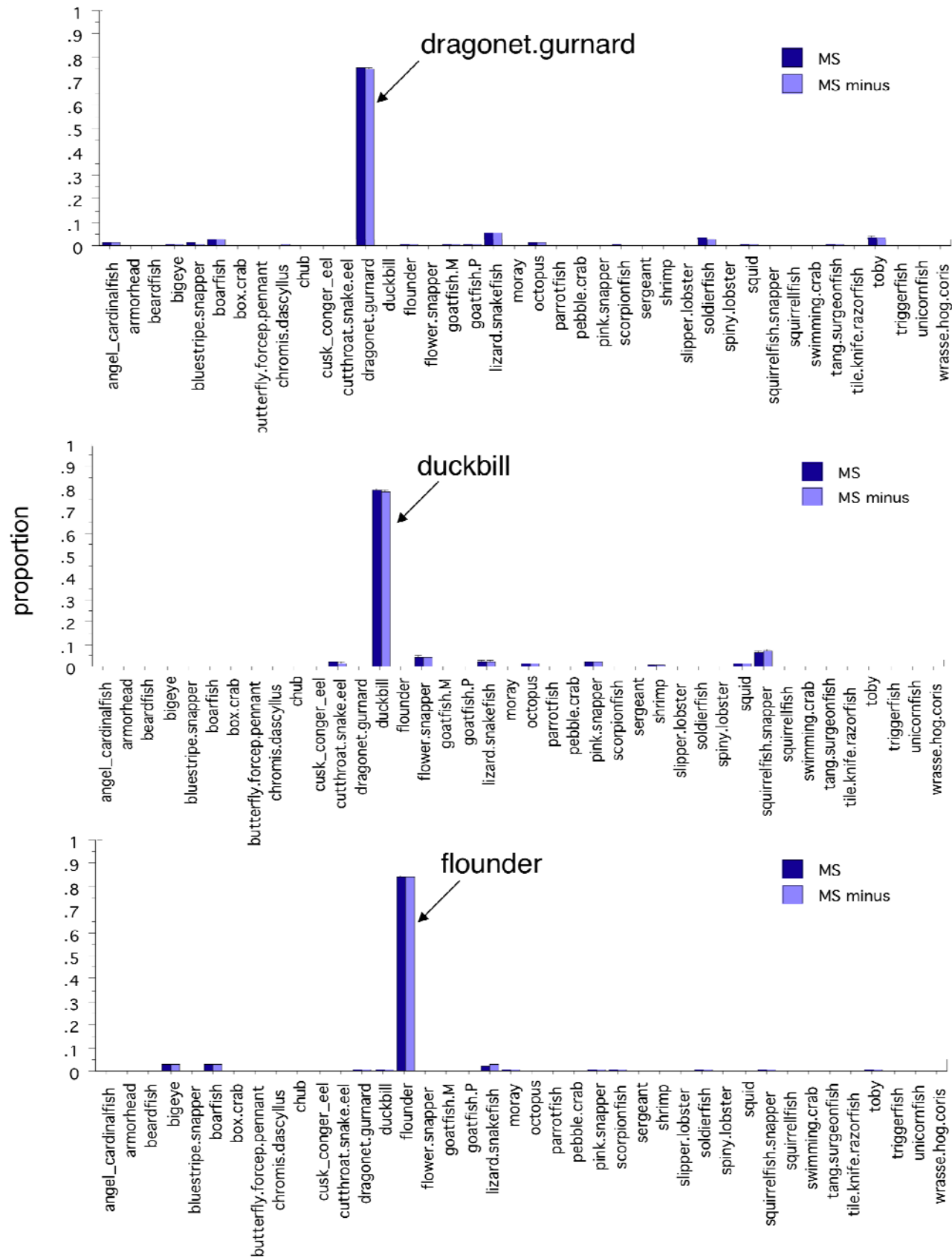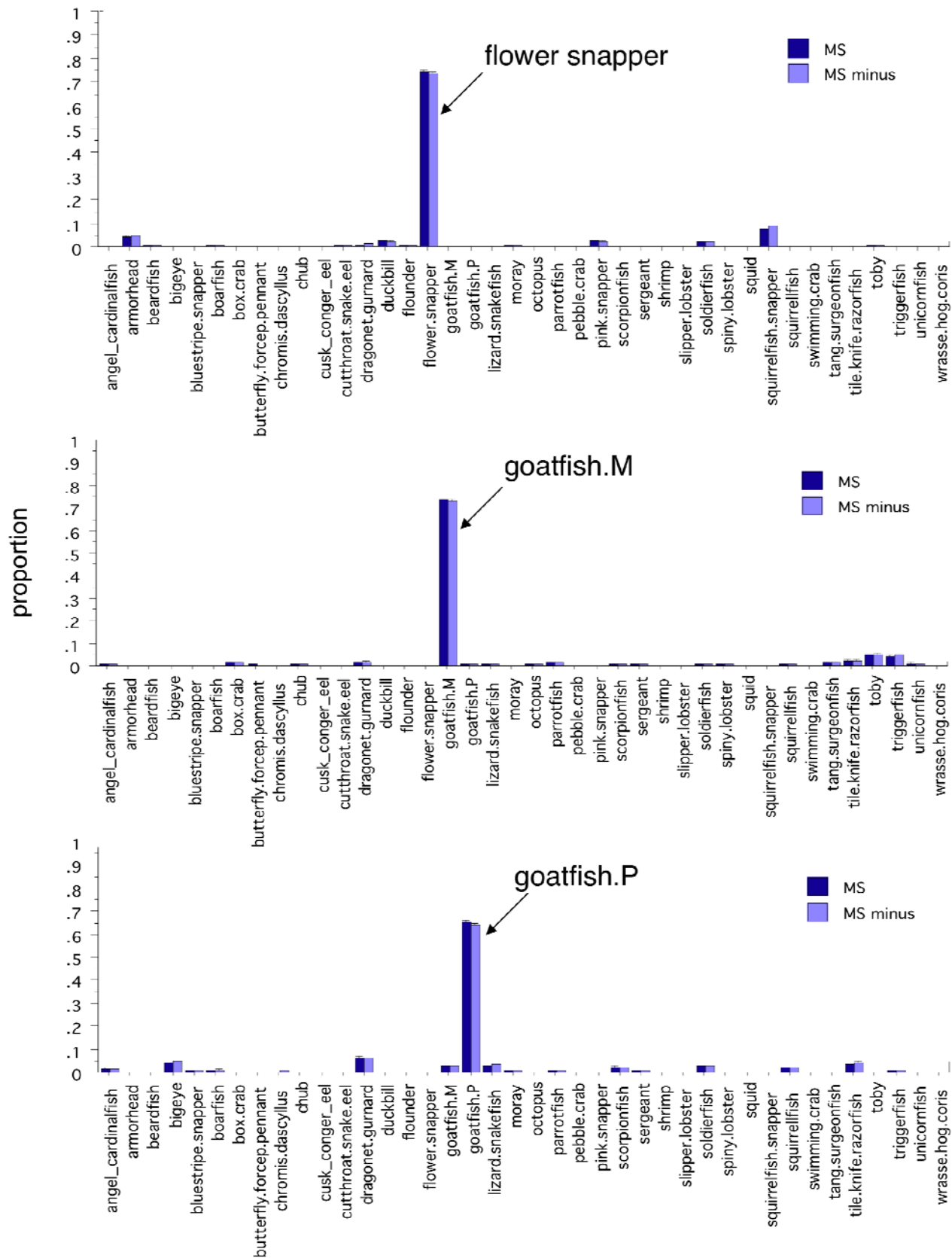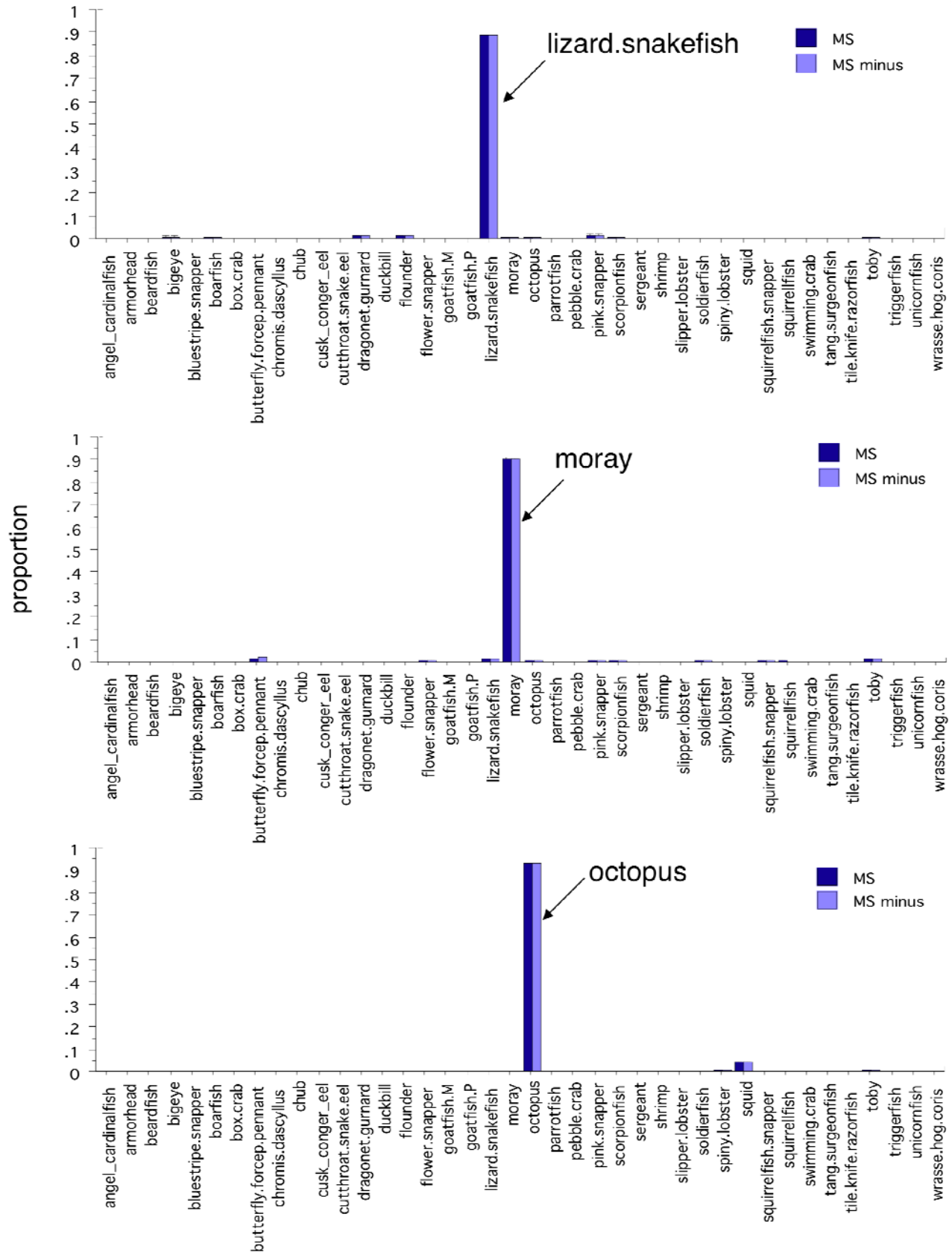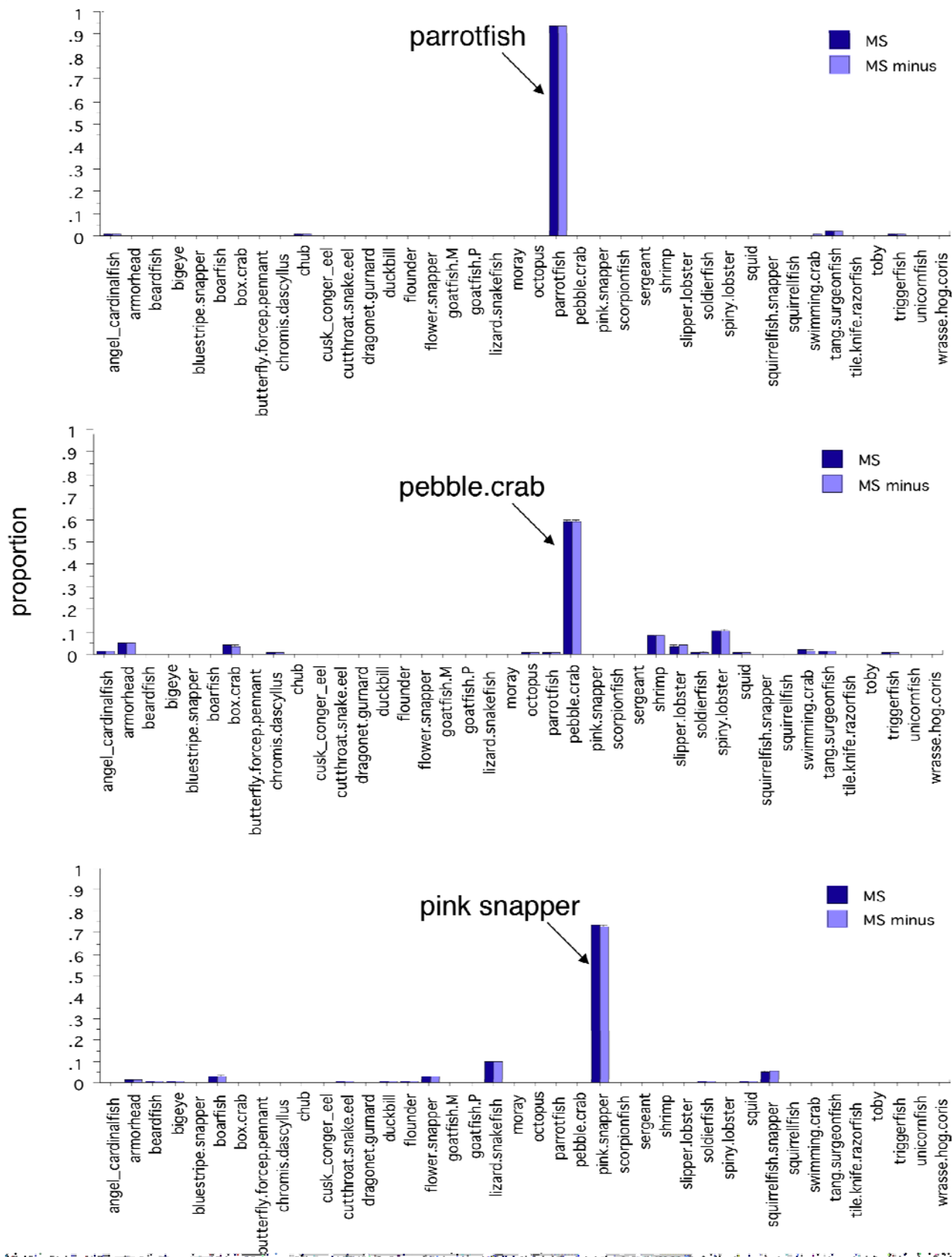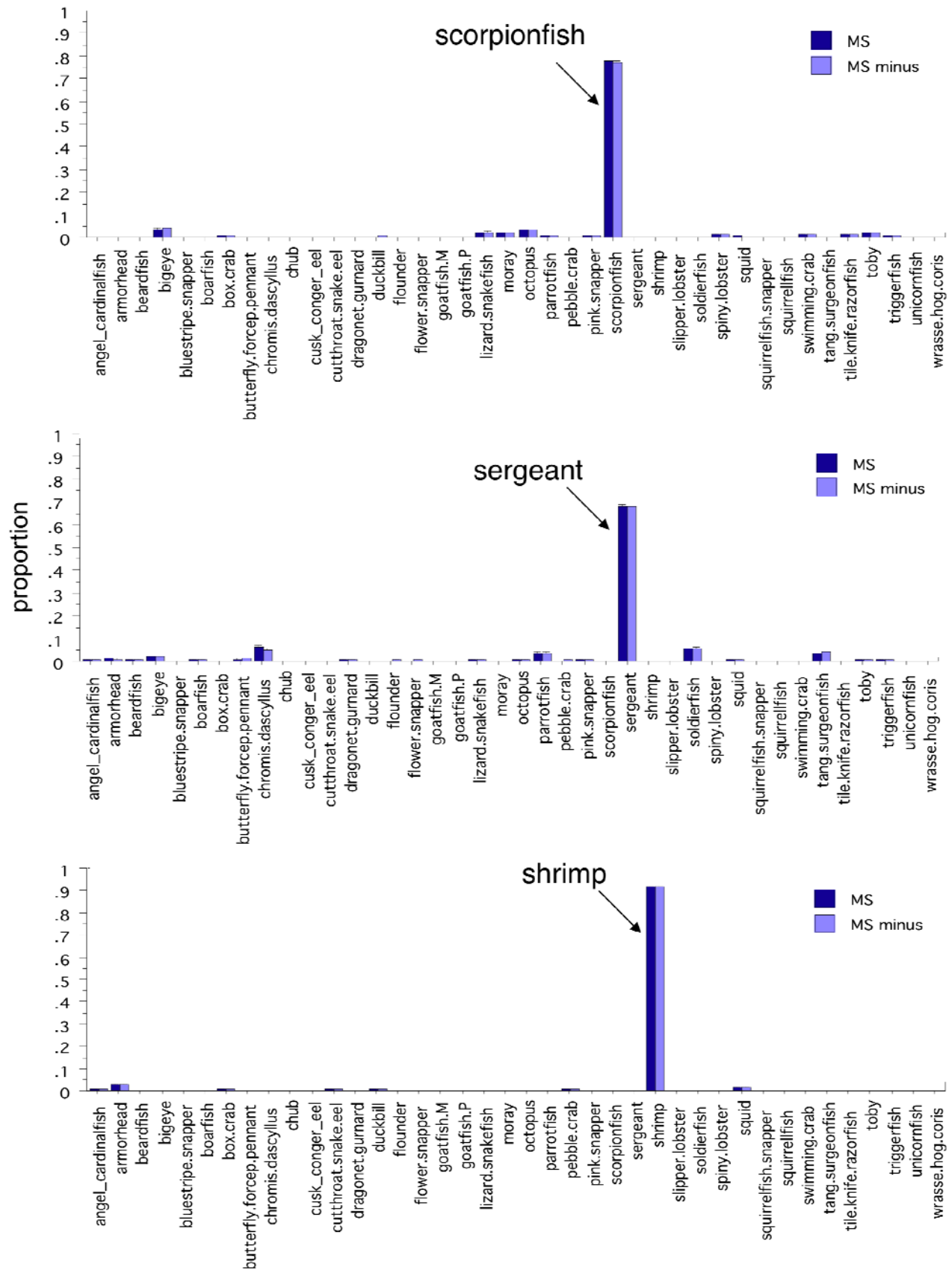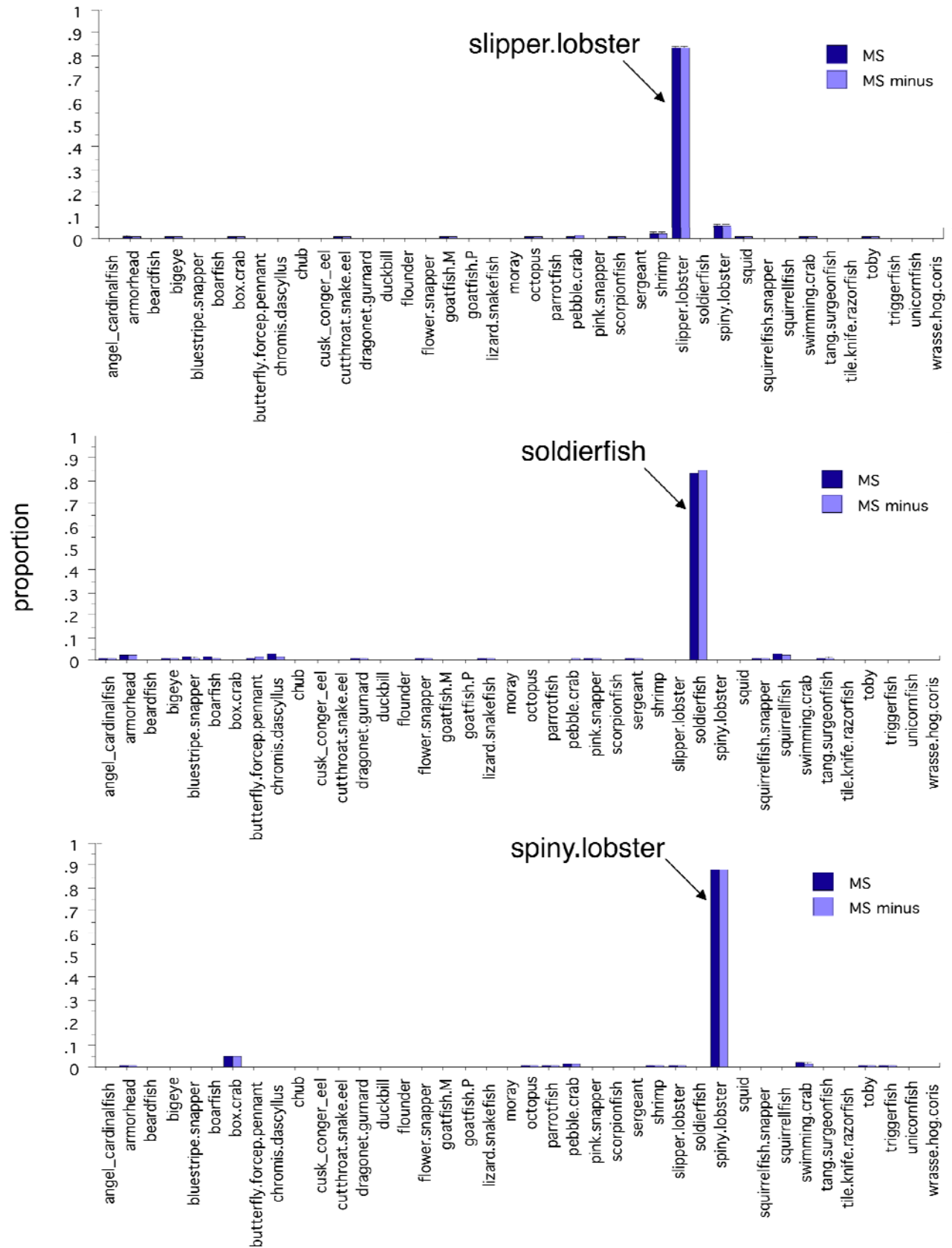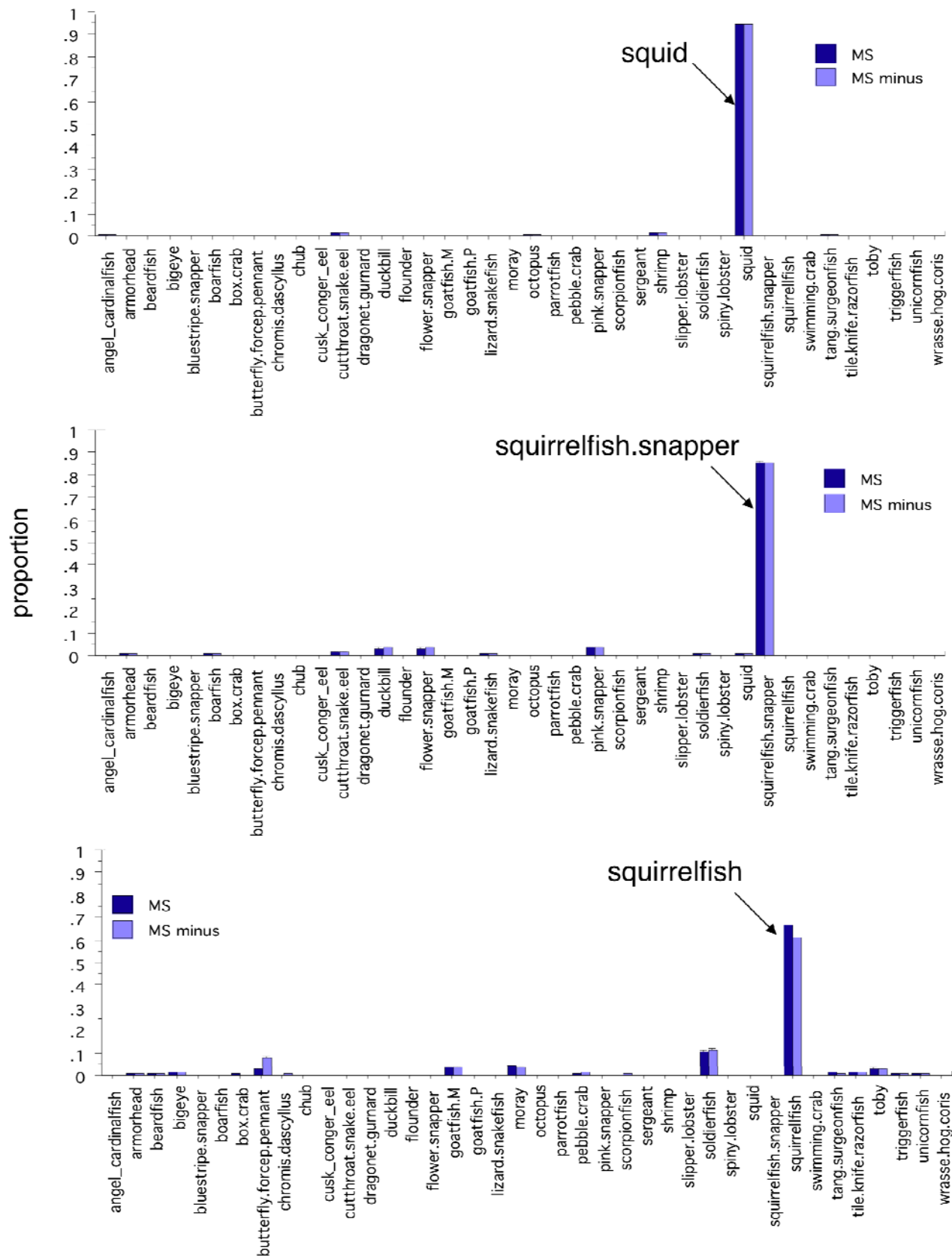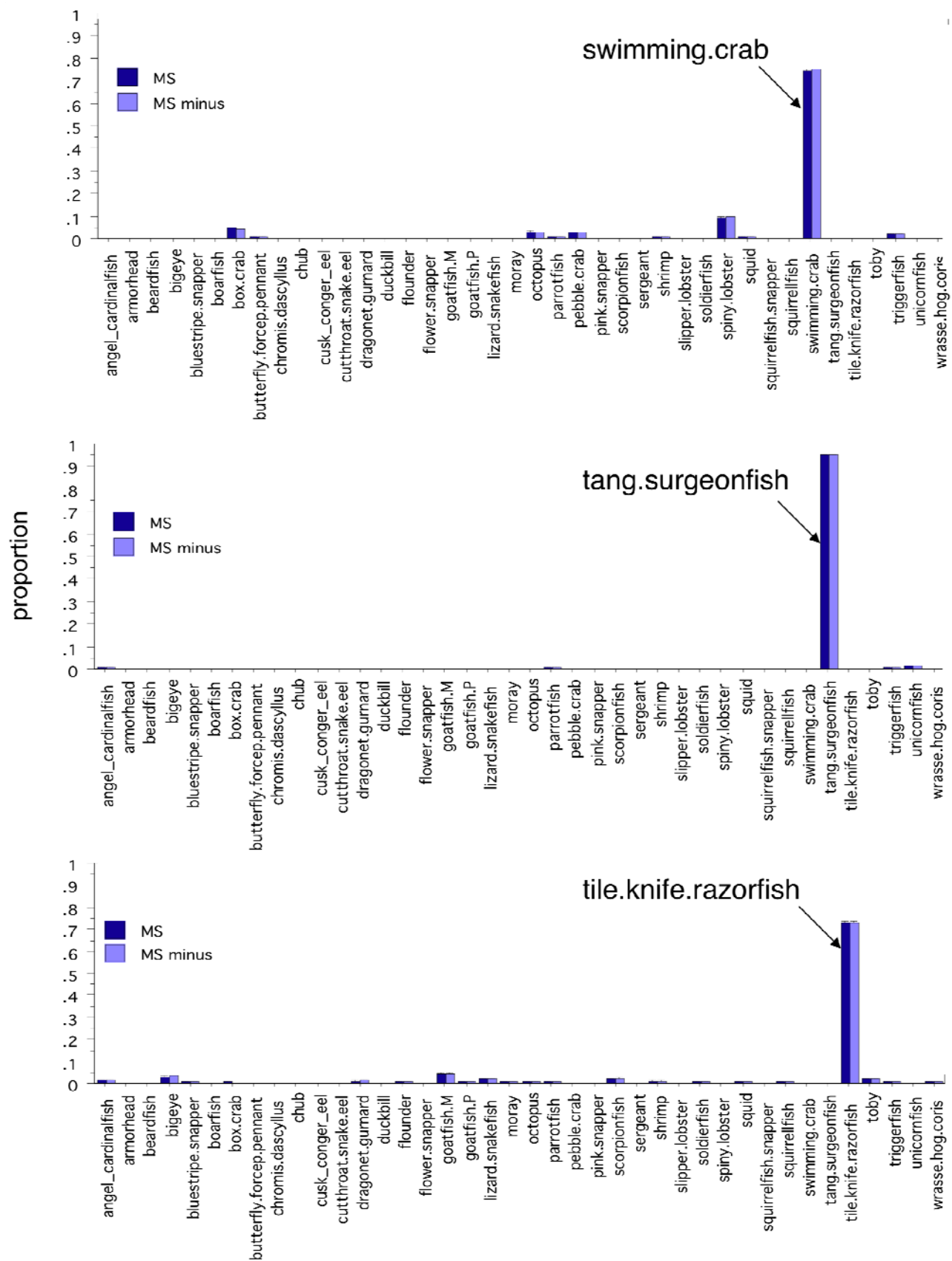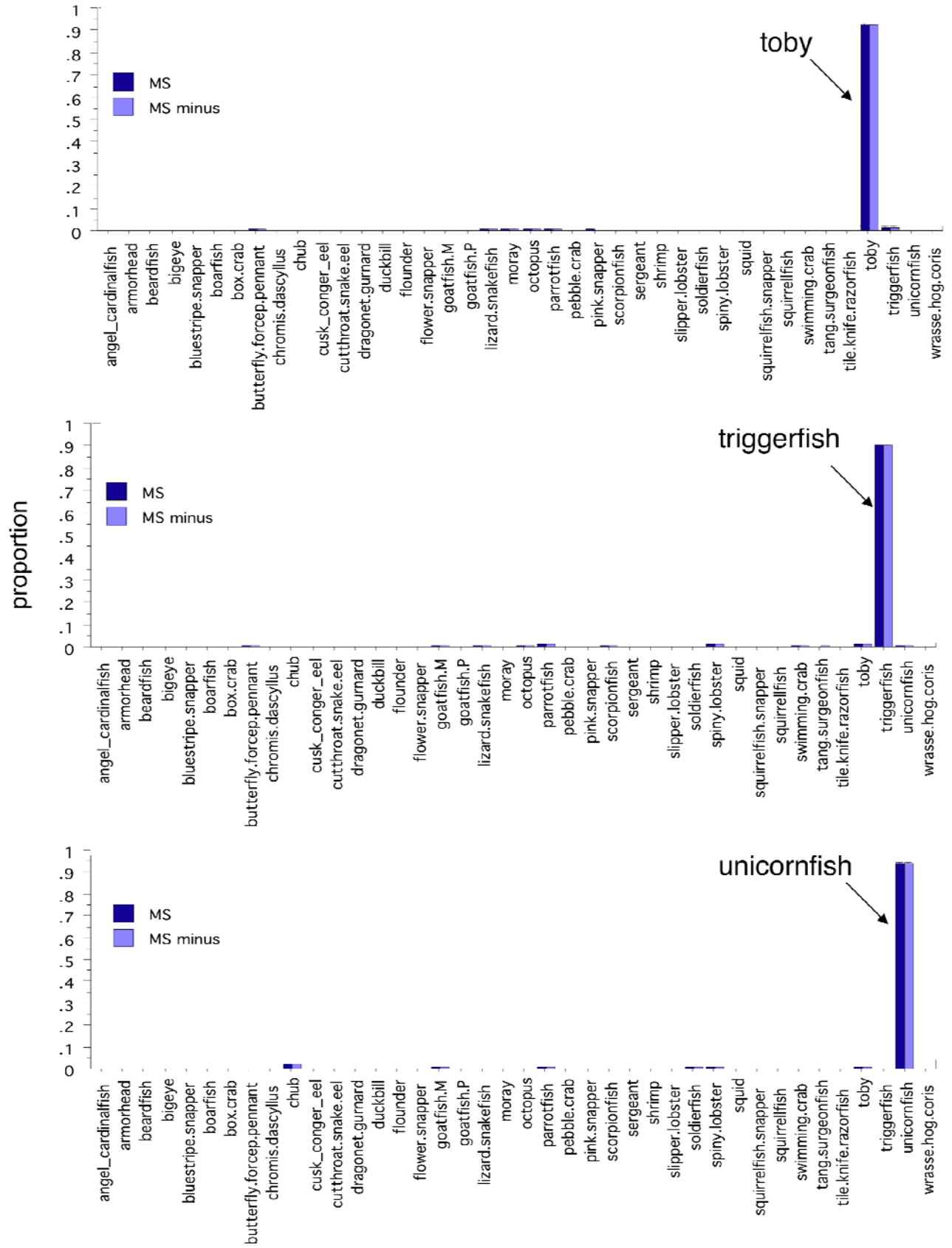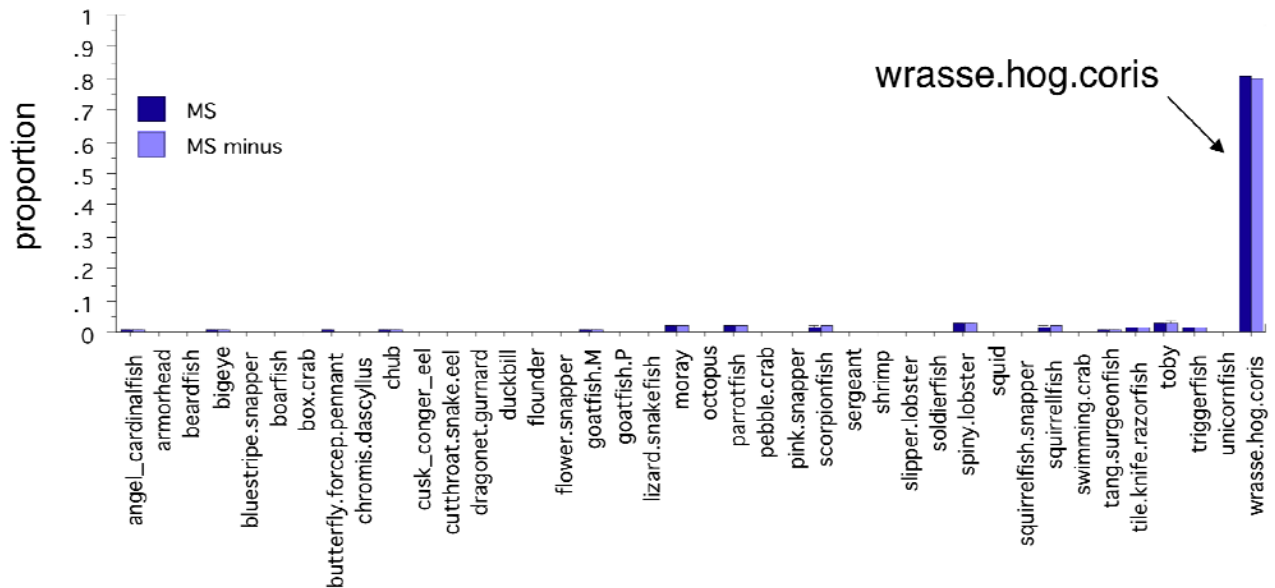
Figure 38.--Each of the 40 plots represents the results of a designated prey species grouping (as labelled) that was modeled on all other prey groupings and itself, and the QFASA estimates of the proportion of each prey grouping estimated as that designated prey. For each designated prey group, the individuals sampled were randomly split into two groups of equal sample size: a simulation group and a modeling group. An average of the simulation group was then modeled in QFASA on the modeling group and all other 39 prey species groups. This process of splitting and modeling was repeated 1000 times. Bars represent means of 1000 runs, and vertical lines represent standard errors (generally too small to see). Each 1000-run modeling series was done with both the MS and MS minus FA set (see Appendix C). The prey group label on each plot is the designated prey group that was modeled and the arrow points to the bar representing the proportion of itself estimated in the QFASA model.

The results illustrated in Figure 38 demonstrate a high overall ability of correctly estimating prey groups in the QFASA model at an average of 78% correctly estimated overall, or > 81% average for all prey groups, after removing three especially problematic groups (beardfish, bluestripe snapper, and chromis.dascyllus). Thus, in general, the model performs well on the 40 prey groups, many times exceedingly well, with some notable exceptions. Prey groups identified and estimated at 90-100% included armorhead, butterfly.forcep.pennant, lizard.snakefish, moray, octopus, parrotfish, shrimp, spiny.lobster, squid, tang.surgeonfish, toby, triggerfish, and unicornfish. Prey groups identified and estimated at 80-90% included bigeye, chub, flounder, slipper.lobster, soldierfish, squirrelfish.snapper, wrasse.hog.coris, and duckbill. By far the poorest estimated were beardfish (34%), followed by bluestripe snapper (43%), and chromis.dascyllus (50%). Pebble.crab was next poorest at 60%, but was almost entirely mistaken with slipper and spiny lobster and some shrimp, thus can to some extent simply be interpreted as crustacean. Four other prey groups (angel_cardinalfish, cusk_conger_eel, goatfish.P, and squirrelfish) were estimated at 64-67%, and the remainder were estimated at > 70-80% (boarfish, box.crab, cutthroat.snake.eel, dragonet.gurnard, flower.snapper, goatfish.M, pink.snapper, scorpionfish, sergeant, swimming.crab, tile.knife.razorfish). While the success rate of estimating species groups overall is fairly good, in all cases, these analyses permit the identification of which species groups may be more similar and thus might overlap somewhat in diet estimates,

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and thus allow some interpretation of modeling results for actual monk seals. These analyses also provide some confidence in the reliability of groupings created to reduce prey species numbers in modeling.

One interesting finding from this series of modeling was that all three deepwater snappers (flower, pink and squirrelfish snappers) were well estimated and did not appear to ever be mistaken for the single shallow-water snapper (bluestripe snapper), while the bluestripe snapper, although poorly estimated, was rarely mistaken for any of the three deepwater snappers. Therefore, we returned to the original discriminant analysis of the carnivore/piscivore cluster (Fig. 12), and identified where these four species were located within the scatterplot distribution. The separation of these two groups of snappers (Fig. 39), suggests some confidence in non-overlap of deep and shallow-water snappers in both discriminant and QFASA analyses.

Canonical Discriminant Functions

1 = Angelfish & Cardinalfish
3 = Beardfish
4 = Bigeye
5 = Cusk & Conger eels
6 = Butterflyfish, Forcepfish & Pennantfish
8 = Sergeant
9 = Damselfish
10 = Dragonet, Helmet gurnard
11 = Goatfish (Mulloidichthys spp)
12 = Goatfish (Parupeneus spp)
13 = Lefteye flounder
14 = Lizardfish & Snakefish
15 = Moray eel
17 = Pufferfish
19 = Scorpionfish
20 = Squirrelfish snapper
21 = Bluestripe snapper
22 = Pink snapper
23 = Flower snapper
24 = Bigscale soldierfish
25 = Hawaiian squirrelfish
28 = tilefish, knifefish, & peacock wrasse
39 = Boarfish
40 = Duckbill

■ = Group Centroid

Figure 39.--Scatter plot of the discriminant analysis on the benthic carnivore and piscivore cluster yielding a cross-validated classification success of 66% (see Fig. 12). Circle **1** encompasses the distribution and group centroid for the single shallow-water snapper (no. 21, bluestripe snapper). Circle **2** encompasses the distribution and group centroids for all three deepwater snappers (no. 23, flower snapper, no. 22, pink snapper, and no. 20, squirrelfish snapper).

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V. EVALUATION OF FA SUBSETS AND CALIBRATION COEFFICIENTS (CCs) IN MODELING DIETS OF FREE-RANGING MONK SEALS USING QFASA

We used this backdrop of the previous and extensive analyses of the NWHI and MHI prey database, to then use QFASA to model the diets of all free-ranging monk seals sampled and analyzed to date (*n* = 248, Table 1) and to interpret these estimates in light of our prey analyses. Here we first evaluate the effect of FA subsets and calibration coefficients (CCs) in model outputs. The CCs used in the QFASA model were derived from the captive monk seal study at SeaWorld as described previously (Section I and Iverson et al., 2010). The variations in these CCs among and within individuals are illustrated in Fig. 40.
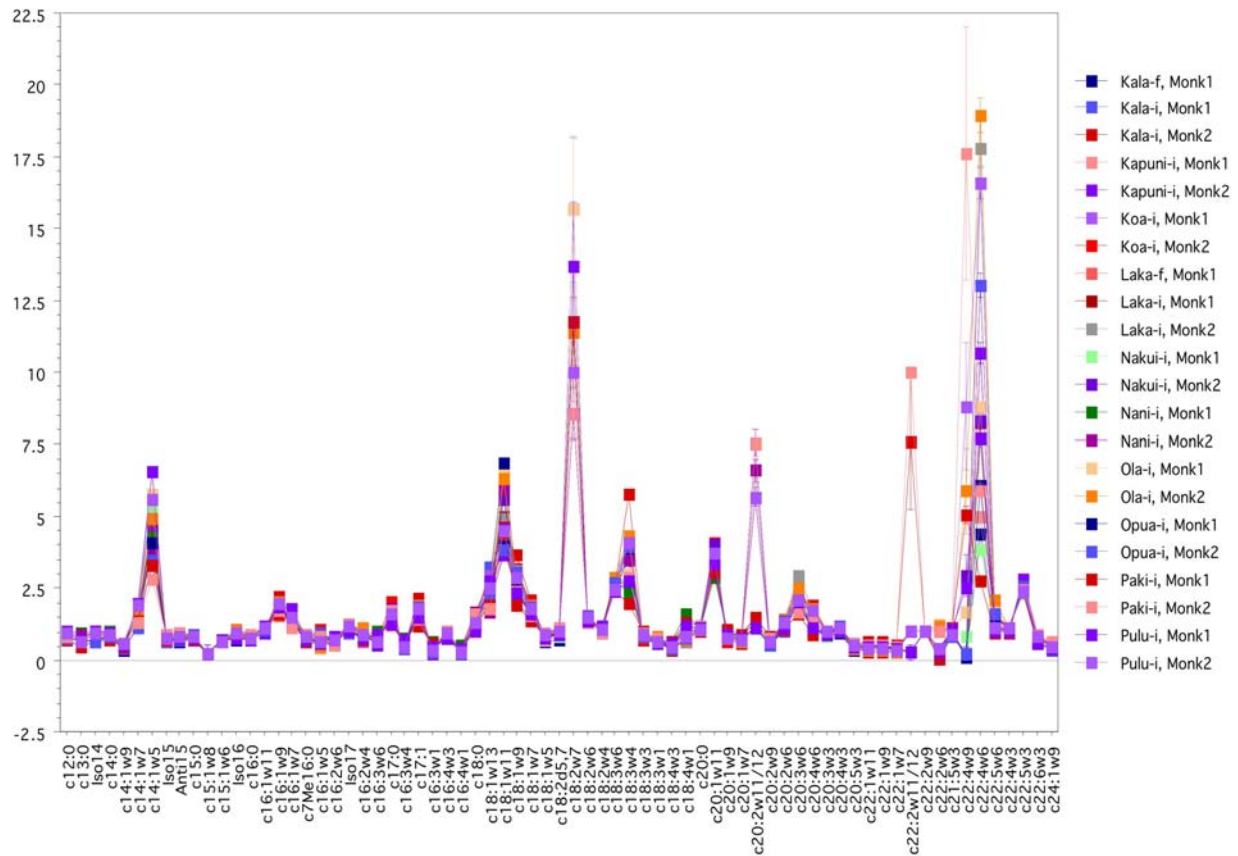
Figure 40.--Individual calibration coefficients (CCs) calculated for each monk seal using several different groupings of herring lots fed ("1" and "2") at SeaWorld San Antonio, and illustrating the very tight grouping for most FAs. The name of the seal with -*i* or -*f* represents the CC for the seal at the beginning (*i*) of the experiment after a long-term (>> year) diet of Atlantic herring or at the end (*f*) of the experiment for the control seals whose diets were not switched during feeding trials.

As described previously, the data for all monk seals and prey in both captive experiments and the wild were carefully evaluated for FAs that were either consistently close to zero in one or both data sets or for those which had highly variable CCs. From this, the subset of FAs was chosen,

which almost exactly matched the modified 1 and 2 extended sets described in the most reliable simulation exercises, but removed any that might be problematic in this ecosystem (see "Prey modeled on other prey" and Appendix C). The monk seal CCs for these FAs were compared with other seal data sets (Fig. 41).
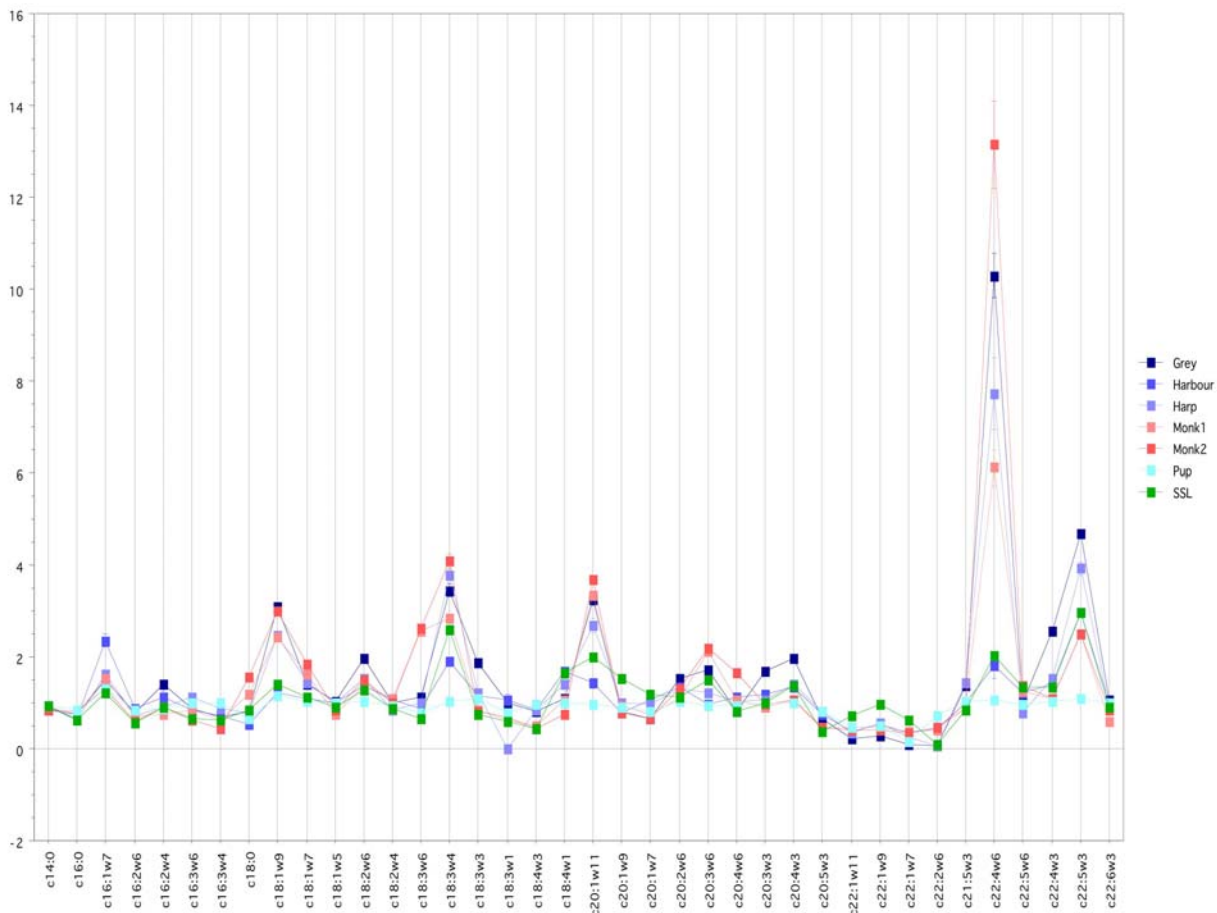
Figure 41.--Final monk seal calibration coefficients (CCs) for non-zero FAs in comparison to those calculated in other studies for grey (adult and pup), harp, and harbour seals and Steller sea lions (SSL) (data from Iverson et al., 2004; S. Iverson, D. Tollit and C. Nordstrom, unpublished data). The only true outlier CC for monk seals was that for 22:4n-6. This FA was measured at quite low levels in the captive study due to a diet of Atlantic herring. However, 22:4n-6 is an abundant dietary FA in the NWHI. Thus, we used several CC values for this FA in modeling the diets of monk seals. Based on a series of analyses of both seals and herring lots, it was concluded that the Monk "1" CCs would be the most reliable as those herring lots fed were most accurately characterized.

As stated above, the FA sets chosen for modeling are listed in detail in Appendix C; however, for ease of the reader, are summarized below in Table 21. Three variations of CCs were also used in modeling: the direct set described in Figure 41 ("direct"), that with the CC adjusted for 22:4n-6 ("CC 22.4", see Table 21), and finally, with adjustments to CCs for any original FA zero values set to 0.0001 before calculating the CCs ("zerosubCC").

Table 21.--FA sets used in QFASA modeling of monk seal diets (see also Appendix C). The "MS" set used the full list; the "MS minus" set omitted those highlighted in yellow. The FA highlighted in red and the box, was also modeled using two different CCs: that calculated from the monk seal captive study (at 8.0) and that calculated from an average of all phocid seal studies (at 2.0). (Note: FAs named as, e.g., 22:4w6 are interchangeable with, e.g., 22:4n-6.)

```
c14.0
c16.0
c16.1w7
c16.2w4
c16.3w6
c16.3w4
c18.0
c18.1w9
c18.1w7
c18.1w5
c18.2w6
c18.2w4
c18.3w6
c18.3w4
c18.3w3
c18.3w1
c18.4w3
c20.1w11
c20.1w9
c20.1w7
c20.2w6
c20.3w6
c20.4w6
c20.3w3
c20.4w3
c20.5w3
c22.1w11
c22.1w9
c22.1w7
c21.5w3
c22.4w6    try with MS CC and also with a CC of 2.0
c22.5w6
c22.4w3
c22.5w3
c22.6w3
```

To test the effects of FA set and CC set on diet estimations, the monk seals were then modeled in QFASA with the following trials:

1) the MS FA set and direct CCs ("directCC MS"),
2) the MS FA set and adjusting the CC for 22:4n-6 to the average for all seal studies at 2.0 ("direct CC 22.4"),
3) the MS FA set and adjustments to CCs for any zero values set to 0.0001 ("zerosubCC MS"),
4) the MS FA set and adjusting the CC for 22:4n-6 to the average for all seal studies and using monk seal CCs with adjustments to CCs for any zero values set to 0.0001 ("zerosubCC 22.4"),
5) the MS minus FA set and direct CCs ("directCCminus"),
6) the MS minus FA set and adjustment to 22:4n-6 ("directCCminus 22.4"),
7) the MS minus FA set and adjustments to CCs for any zero values ("zerosubCCminus"), and
8) the MS minus FA set and adjustments to CCs for any zero values with adjustment to 22:4n-6 ("zerosubCCminus 22.4")

Figure 42 illustrates the estimated distribution of diet in all seals across all 40 prey groupings using the eight variations in model parameter (FA and CC set) used, as described above. Perhaps surprisingly, there was little difference in diet estimates for any of the model inputs that varied the FA or CC set used. At the very most, there was occasional trace appearance and disappearance of minor prey, and with means of major prey varying only slightly. Given that the results were all comparable, and that we had no *apriori* reason to choose one model output over another, we averaged across all eight models for each individual seal.

The direct output of the QFASA model is the relative contribution of prey FA signatures to the overall signature of the predator. In order to translate those proportions into relative contribution to diet, the estimated signature contribution from prey must be corrected to account for differences in fat content (and thus FA contribution) among prey types. All else being equal, species with a higher fat content will contribute proportionately more to the predator signature than those with a lower fat content. However, given that we know the fat content of each prey (see Appendix B), it is straightforward to translate the estimated signature contribution to the proportion of each prey type eaten. The QFASA model now has incorporated a direct "diet" output, which automatically accounts for variation in prey fat content. However, an interesting note is that while most prey in the NWHI tend to be relatively low in fat, the amorhead stands out at a remarkable ~ 25% fat. Thus, as illustrated in Figure 43, although the diet estimate for this prey is very low (< 1% of diet), because of its very high fat content, this low intake is estimated to contribute to > 6% of monk seal fat stores overall.

It should be noted that for each individual seal, the QFASA model derives an estimate for the proportion of a given prey in its diet, a standard error for the estimate of that prey, and a lower and upper confidence limit for the estimate of that prey – for each prey grouping within each individual seal. These are contained in fairly large spreadsheets and are available upon request. For the purposes of this report, we focus on summarizing the current overall diet estimates. Nevertheless, Figure 44 illustrates the average upper confidence limits of each prey estimated among individuals.
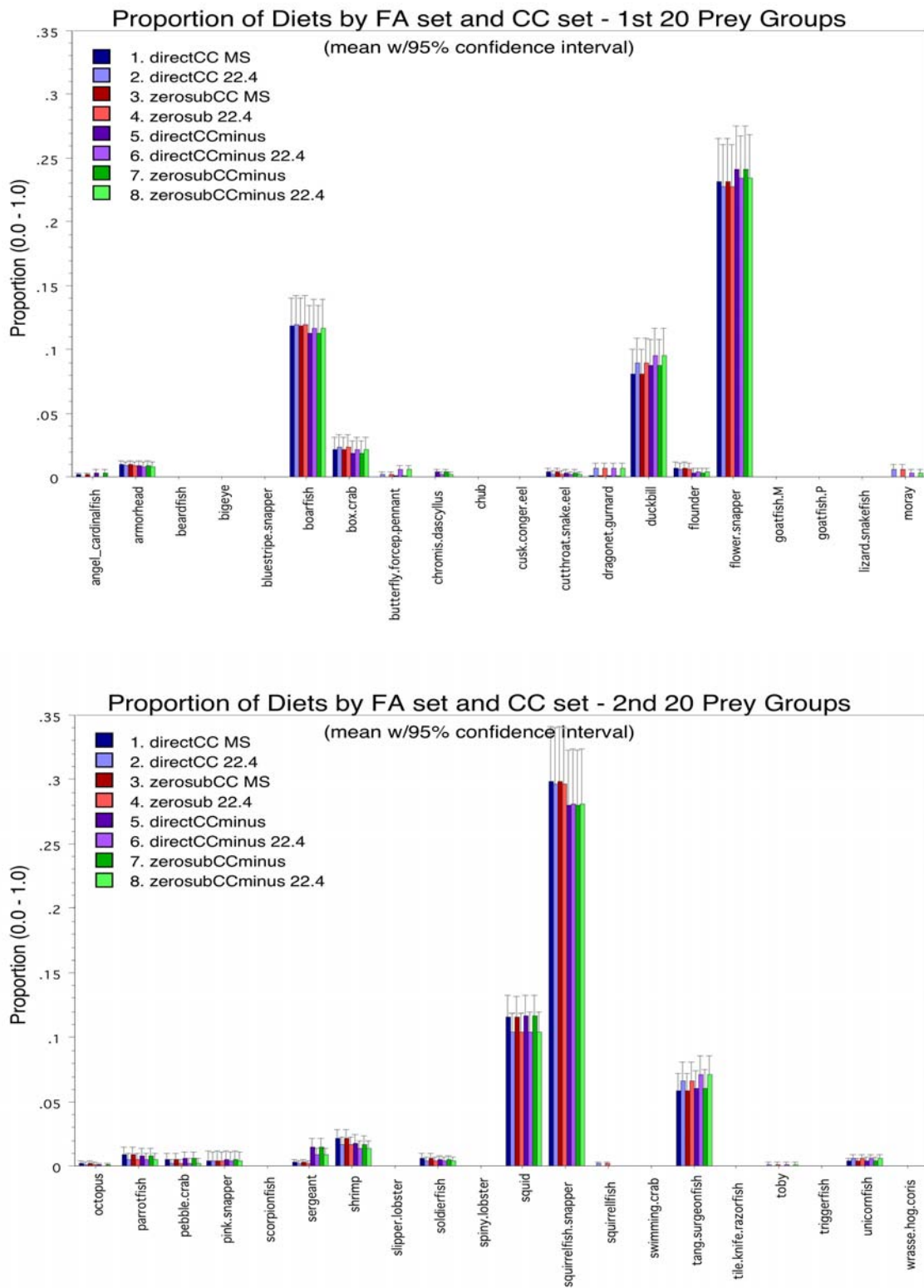
Figure 42.--Proportion of prey in estimated in diets of monk seals using the 8 variations in model inputs. First and second 20 of the 40 prey groups are illustrated in the two graphs to more clearly see results.
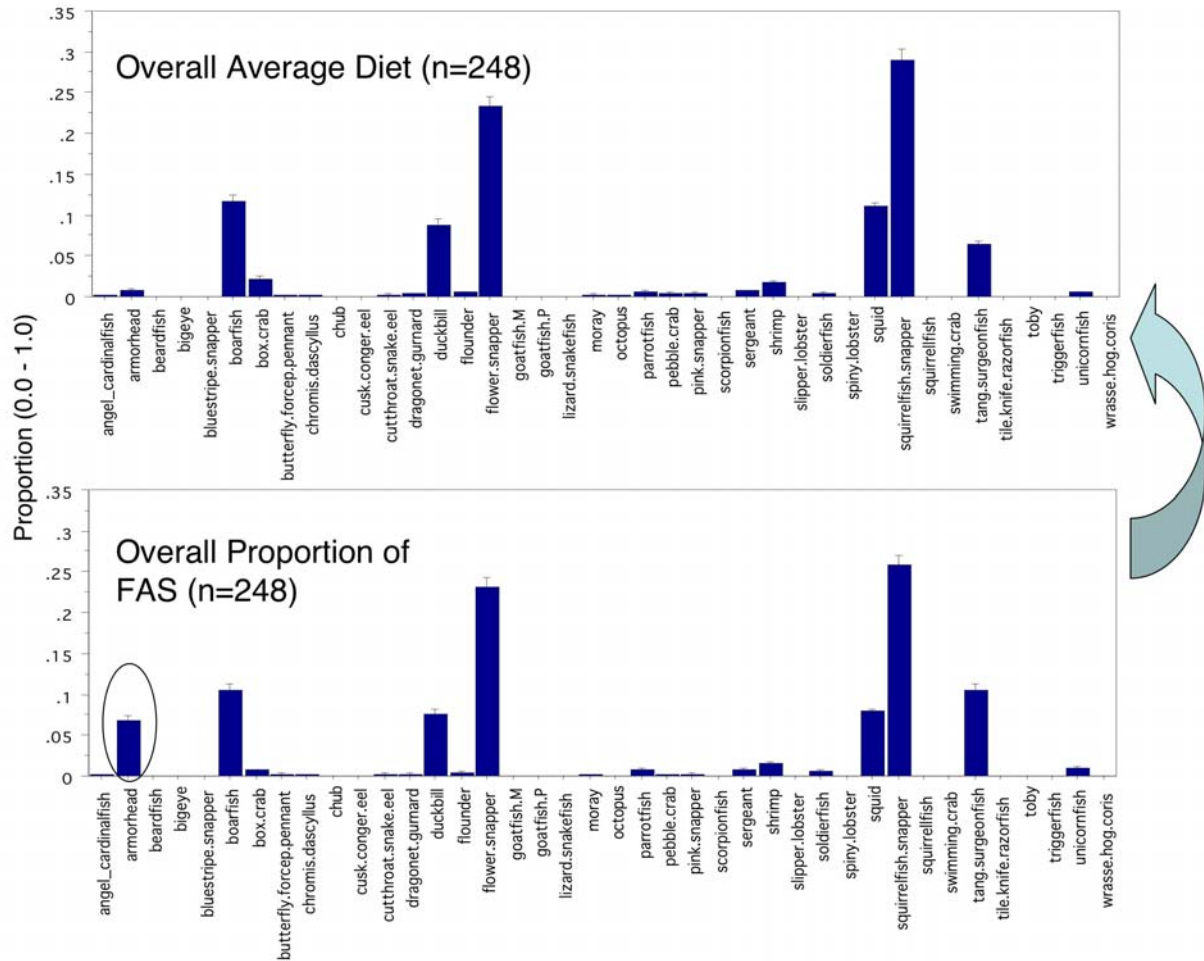
Figure 43.--Proportion of prey estimated in the FA signatures of monk seals (bottom) compared to how this translates to proportion of diet (top) after accounting for differences in fat content (Appendix B) among prey. Armorhead (*Pseudopentaceros wheeleri*) is circled given its unusually high fat content (~ 25%).
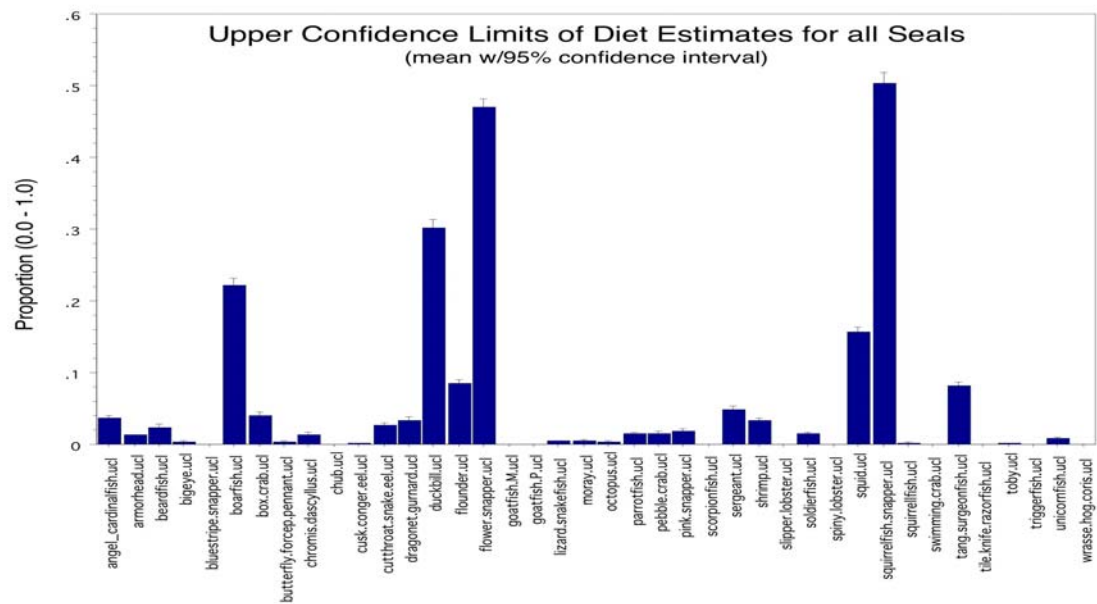
Figure 44.--The average upper confidence limits of each prey estimation among all individuals.

VI. ESTIMATES OF MONK SEAL DIETS IN THE NWHI AND MHI USING QFASA

Diet Estimates of Individual Monk Seals

For each individual monk seal sampled (Table 1), diets were estimated using the procedures specified above (Section V). Some seals were sampled more than once during the course of this study and provide insight into both individual variability and consistency (Fig. 45).
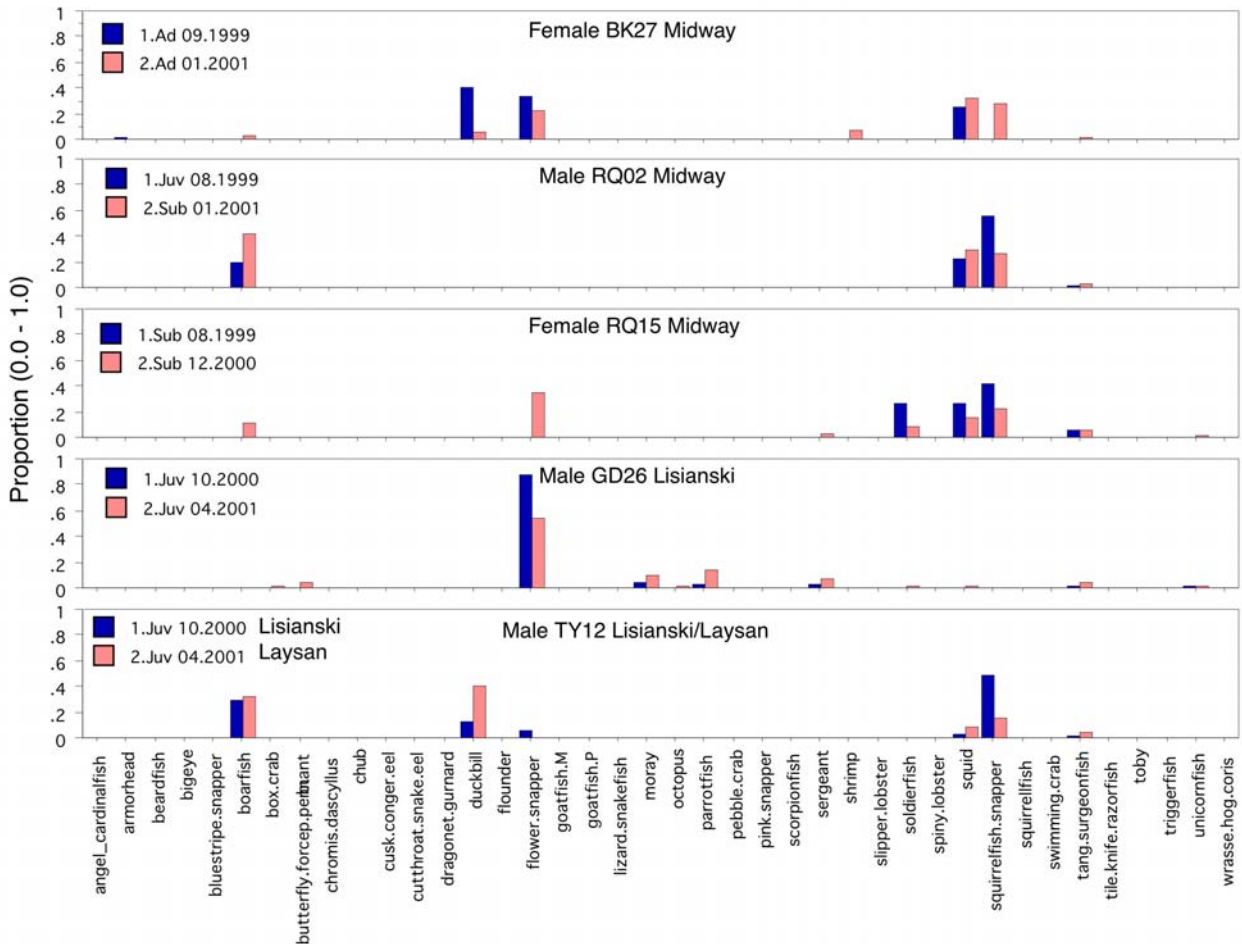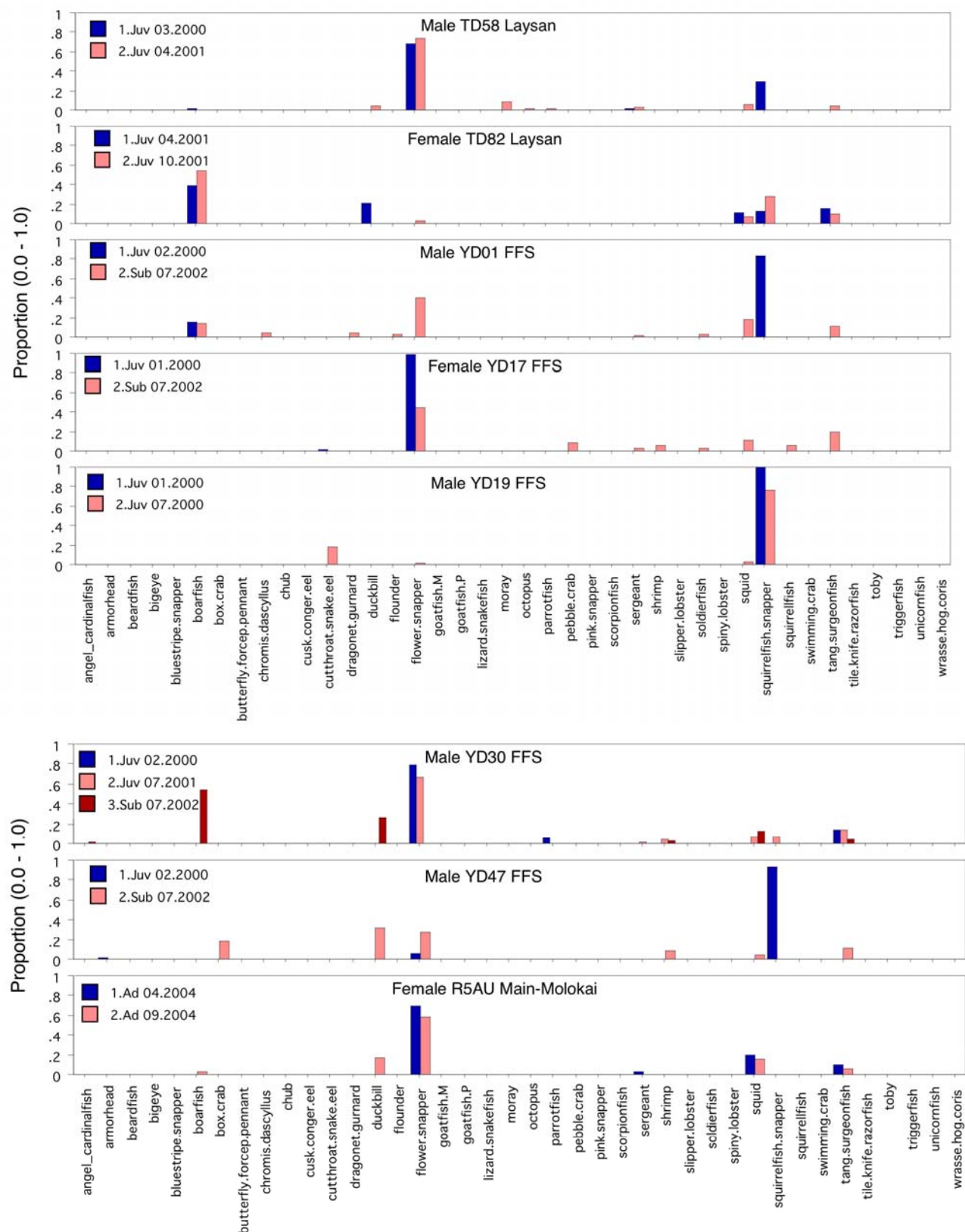
Figure 45.--Proportions of prey estimated in diets of repeat-sampled monk seals. The legend refers to the age-class the animal was classified as, followed by the month.year of sampling.

Individual diets comprised of a mixture of species and in general, although somewhat variable, diets tended to be similar within individuals but differed among individuals. The largest differences that were found within an individual were particularly when a seal was sampled as a juvenile and then later as a subadult (Fig. 45).

Overall Diet Estimates by Demographic Group and Location

Across all locations sampled in the NWHI and MHI, monk seal diets were estimated to be comprised of a mixture of species, the most abundant of which were boarfish, duckbill, box.crab, flower snapper, shrimp, squid, squirrelfish snapper, and tang.surgeonfish. However, estimates varied widely among age groups, with adults and subadults estimated to consume higher levels of boarfish, box.crab, duckbill, squid and tang.surgeonfish, in contrast to juveniles who were estimated to consume much lower levels of some of these prey and higher levels of flower snapper, parrotfish and squirrelfish snapper (Fig. 46). When further subdivided by sex within age-class, differences between adults and juveniles remained apparent, but also suggested differences in diets of adult males and females (Fig. 47). These results were consistent with previous findings of qualitative differences in diets among demographic groups from discriminant analyses (Figs. 5, 6).
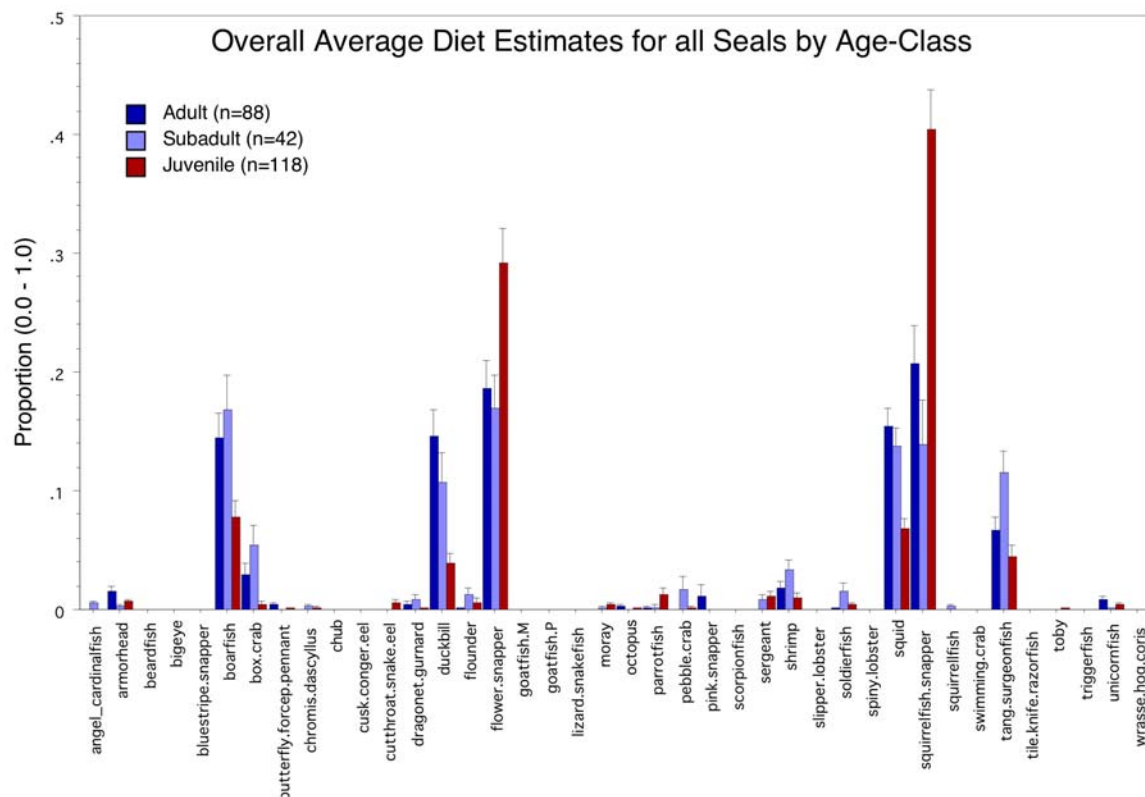
Figure 46.--Proportions of prey estimated in the diets of monk seals across all regions. Bars are means and vertical lines are 1 standard error.
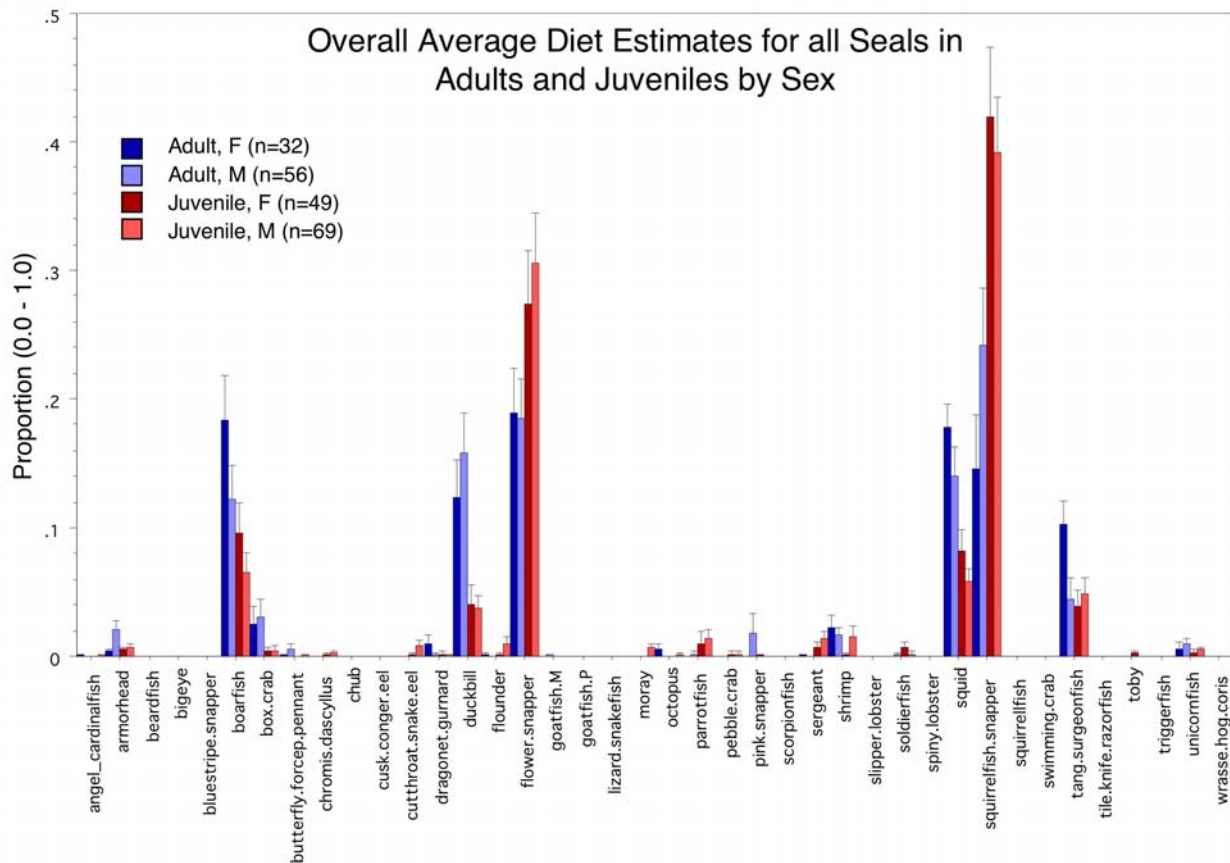
Figure 47.--Proportion of prey estimated in the diets of monk seals across all regions. Bars are means and vertical lines are 1 standard error.

Averaged across all monk seals sampled in all regions, diets of adults were dominated in almost equal amounts by boarfish, duckbill, flower snapper, squid, squirrelfish snapper and tang.surgeonfish. In contrast, juvenile diets were estimated to be heavily dominated by the two deepwater snappers (flower and squirrelfish snappers); more minor inputs were apparent from boardfish, duckbill, squid, and tang, surgeonfish (Fig. 47).

Within each region of the NWHI, diets as a whole varied, as illustrated in Figure 48. Although the same prey groups appeared to be abundant across regions, their absolute levels varied by region, consistent with previous discriminant analyses (Fig. 8). When the diets of monk seals sampled in the NWHI were compared to those sampled in the MHI, several differences appeared (Fig. 49). Moving from westernmost to easternmost (MHI), the proportion of boarfish, duckbill, squid and squirrelfish snapper became steadily reduced (especially in the MHI), while the proportions of flower snapper and tang.surgeonfish steadily increased (Fig. 49).
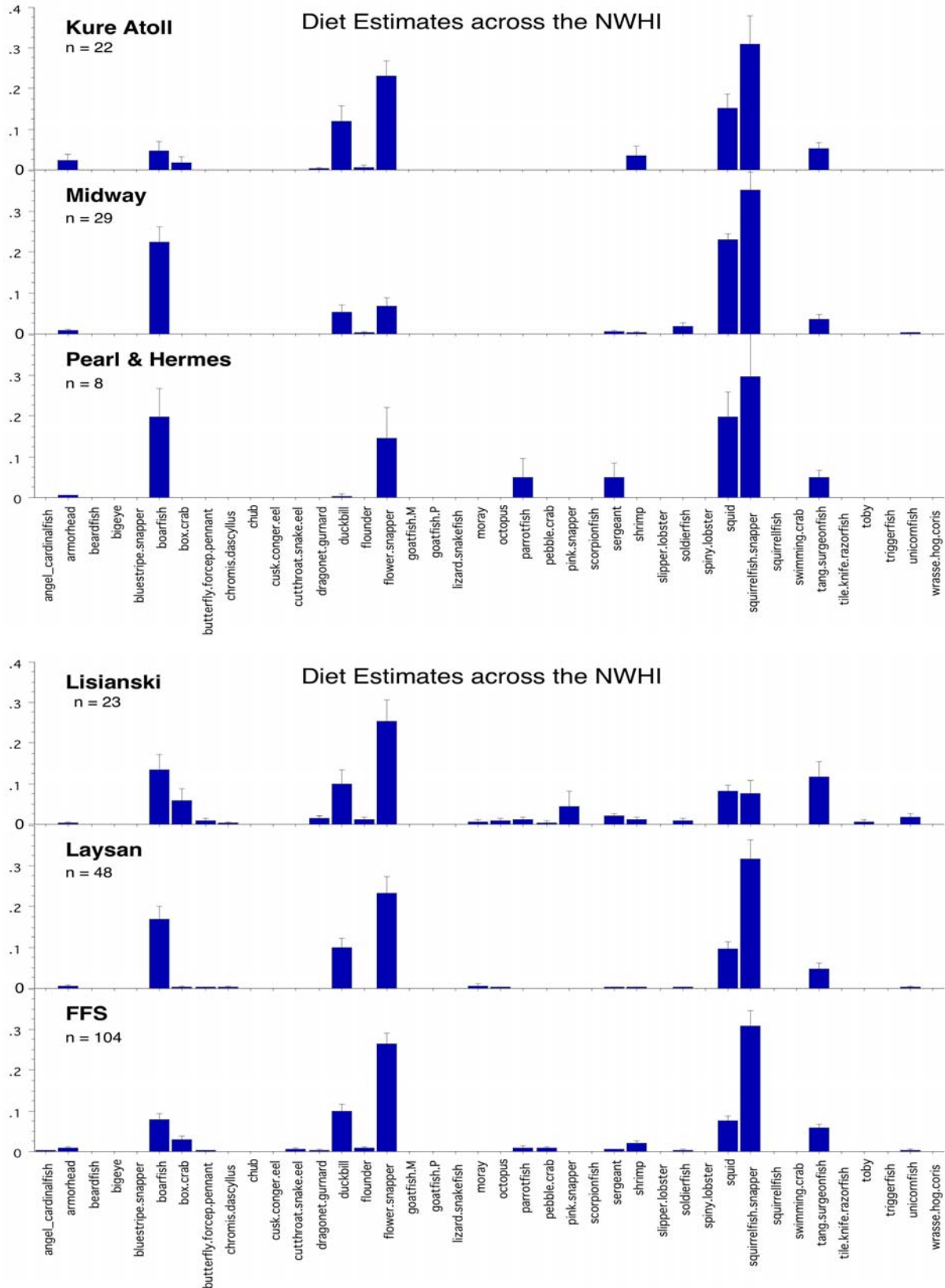
Figure 48.--Proportion of prey estimated in the diets of monk seals within each region of the NWHI. Bars are means and vertical lines are 1 standard error.
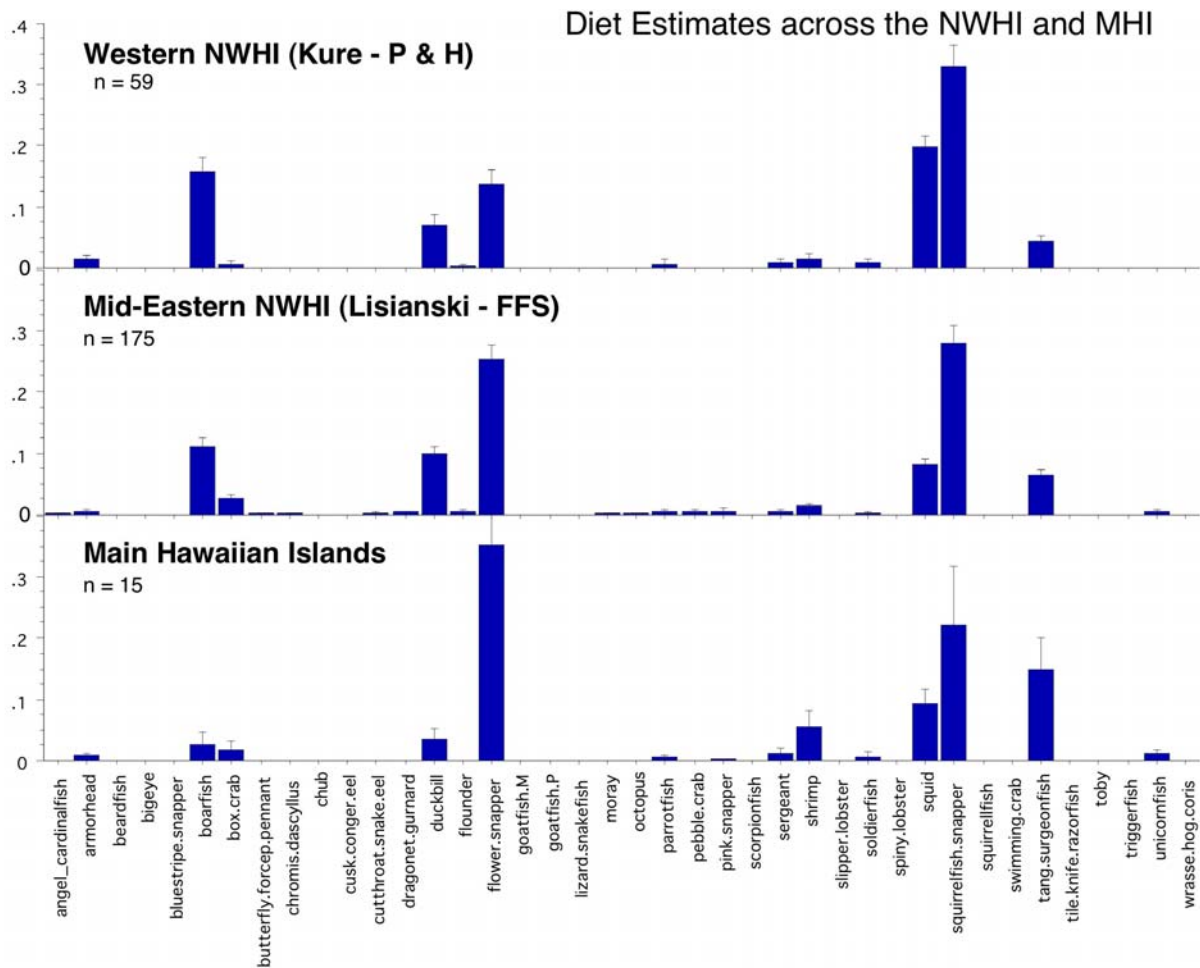
Figure 49.--Proportion of prey estimated in the diets of monk seals across major areas of the NWHI and in the MHI. Bars are means and vertical lines are 1 standard error.

Given that relatively few monk seals were sampled in the MHI, we just examined the differences in diets by age and sex class within the major regions (see Fig. 7) of the NWHI (Fig. 50). While a large degree of variability in diets was estimated across regions and among demographic groups, diets of juveniles were still dominated by the two deepwater snappers, with varying contribution from an array of other prey. Diets of adults and subadults remained characterized by a relatively consistent array of prey, although results suggest a large degree of individual variation (Fig. 50).
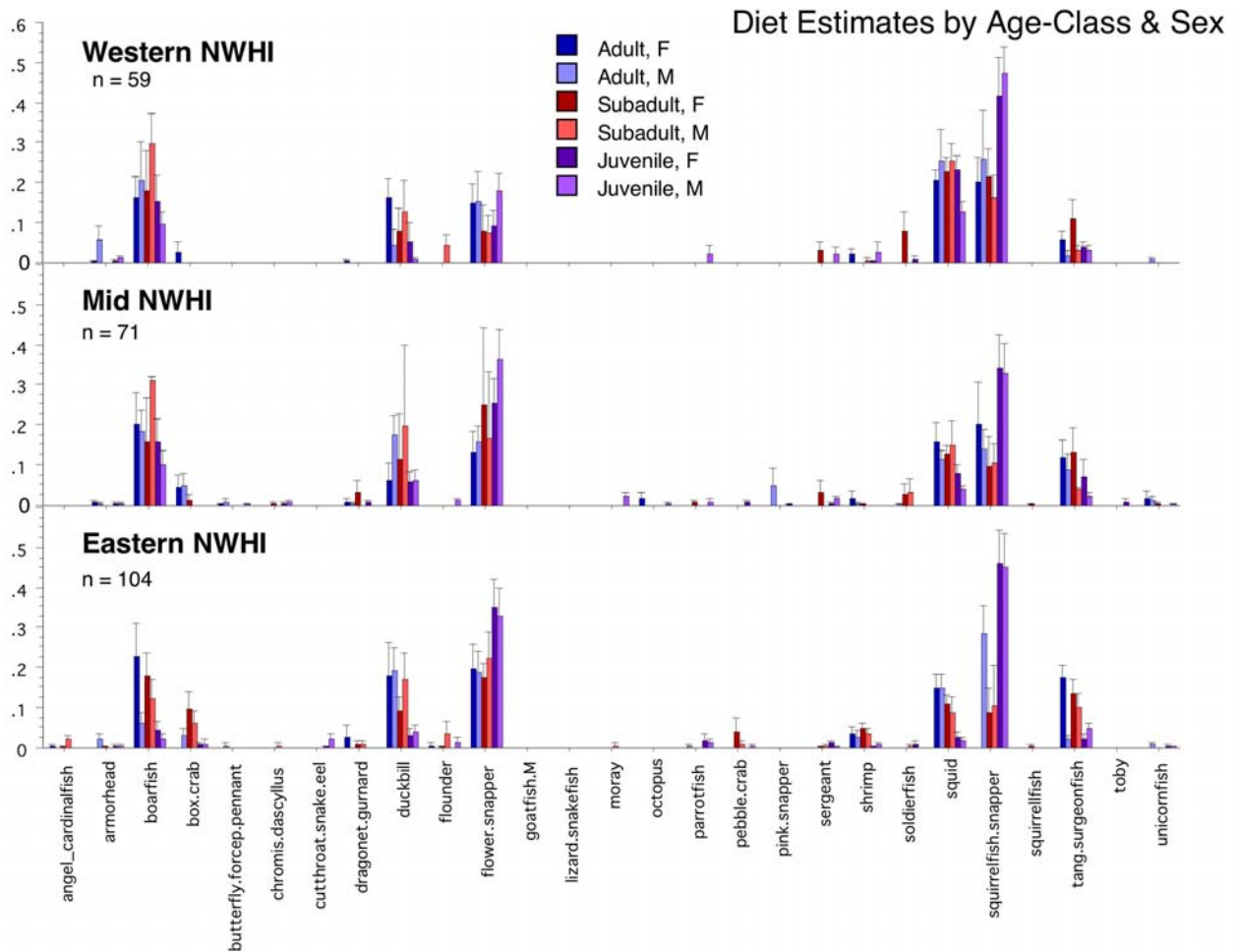
Figure 50.--Proportion of prey estimated in the diets of monk seals across major areas of the NWHI (see Fig. 7) separated by age-class and sex; only species consistently identified in diets are included. See Table 1 for individual sample sizes of demographic groups. Bars are means and vertical lines are 1 standard error.

Finally, although the preceding plots suggest some consistency in the identification and estimation of prey important to monk seals and age-classes on average, indeed few single seals possessed the "average diet". To provide a sense of this variation, Table 22 presents the maximum value for each prey group estimated in an individual seal within the NWHI. For example, while armorhead was estimated to comprise only a small fraction of diet (0.9%) on average, it occurred as high as 27% in one adult and 11% in a juvenile. Likewise, box.crab appeared consistently across groups and regions in relatively minor proportions (2.1%), but was found as high as 26-55% of an individual's diet. The deepwater snappers made up almost 100% of several individuals' diets. Octopus and squid appeared as high as 11% and 65% of the diet in an individual, respectively although they averaged 0.1% and 11% overall, respectively. Several species did not contribute significantly to estimated diets, including cusk_conger_eels, goatfish, lobsters and wrasses.

Table 22.--The maximum portion of each prey group in the NWHI to be estimated in an individual monk seal within demographic groups.

Prey Group	Average across all seals	Max-adult	Max-subadult	Max-Juvenile
angel_cardinalfish	0.1	3.1	8.4	3.6
armorhead	0.9	26.6	4.6	10.6
beardfish				
bigeye				
bluestripe.snapper				
boarfish	11.7	77.2	65.7	55.1
box.crab	2.1	54.8	44.6	26.1
butterfly.forcep.pennant	0.2	11.5	1.4	4.5
chromis.dascyllus	0.1	0.3	4.5	9.4
chub				
cusk_conger_eel				
cutthroat.snake.eel	0.3			18.9
dragonet.gurnard	0.4	17.0	12.7	8.6
duckbill	8.8	81.4	45.3	50.5
flounder	0.5	3.9	24.3	35.5
flower.snapper	23.3	76.9	80.8	98.6
goatfish.M	0.0	5.1		
goatfish.P				
lizard.snakefish	0.0		0.1	
moray	0.2	1.6	4.8	16.1
octopus	0.1	11.1		5.9
parrotfish	0.7	8.0	2.8	45.1
pebble.crab	0.4	1.7	47.4	12.7
pink.snapper	0.4	89.1		3.4
scorpionfish				
sergeant	0.7	1.4	11.9	27.7
shrimp	1.7	27.7	17.9	48.3
slipper.lobster				
soldierfish	0.5	6.0	26.3	22.2
spiny.lobster				
squid	11.0	65.3	34.2	42.6
squirrelfish.snapper	28.9	92.6	93.7	99.9
squirrellfish	0.1	1.4	5.7	0.7
swimming.crab				
tang.surgeonfish	6.4	83.2	45.0	58.1
tile.knife.razorfish				
toby	0.1	1.6		10.4
triggerfish				
unicornfish	0.5		1.3	9.9
wrasse.hog.coris				

VII. CONCLUSIONS AND SUMMARY

Our aim has been to determine whether aspects of monk seal foraging could be inferred qualitatively using FA signatures of blubber stores and whether monk seal diets could be estimated using QFASA. Without question, the former has been confirmed both through captive study and application to free-ranging animals (Figs. 1, 2, 5, 6, 8). QFASA was originally developed to allow the quantitative estimation of predator diets, integrated over time, from the FA signatures of their lipid stores and a comprehensive prey database. The performance of QFASA has been validated in a number of predator species, as discussed previously. While monk seals appear to be similar to other seals (and mammals in general), in terms of how they metabolize and deposit FAs (e.g., Figs. 1, 2, 40, 41), the ecosystem within which they live presents extremely difficult issues for QFASA, arising from the fact that the number of potential prey species is huge, in the order of some 200, many of which share similar habitat and feeding ecology with one another, which will thus result in some similar FA signatures among closely tied diet guilds. In addition, the QFASA model cannot deal with these large numbers, both from a computational standpoint and from the issue of modeling on many more species than FAs. Thus, there was no option, but to reduce the overall number of prey for modeling, by grouping species into supertaxa. After a number of tests of grouping procedures, we concluded on the one we have employed in this current series of analyses. From both discriminat analyses, and especially the comprehensive simulation studies, results suggest that our groups are not only ecologically meaningful, but are generally performing well.

In terms of distinguishing prey groupings from all other 40 prey groupings in the simulated diets, in most cases the major prey species in the specified diets were identified and many times estimated at their specified levels fairly well. This is encouraging, given the huge complexity of this prey database and ecosystem. If, in the modeling of actual monk seal diets, the model performs as well or better than in simulations, then it would mean that QFASA is, overall, detecting the major prey that monk seals are utilizing. However, although analyses such as those depicted in Figures 35 and 36, provide overall confidence in identifying prey rather well, one has to be very aware, in this ecosystem – which is pushing the very limits of QFASA, of several important issues that remain: 1) that of overlap in some problematic species, as well as 2) noise in terms of false positives. The second issue may be easier to deal with. As illustrated by the simulation graphs in general, species that appear in actual diet estimates at very low or trace levels may have to be taken with a grain of salt; that is, while minor occurrences in diets could be real, such levels may also reflect noise. However, if such prey are indeed very minor in monk seal diets in any case, then they likely would not matter significantly to the monk seal population in general. The more significant issue to be aware of, is which species may be exhibiting most overlap with one another such that it results in the appearance of significant false positives and underestimation of actual prey of importance - and to then determine how best to deal with those prey in terms of interpreting modeling results for actual monk seal diet estimates. The pseudo seal diet simulations provided important insight into this.

However, perhaps the tests of modeling prey on other prey are more directly revealing (Figs. 37 and 38). These studies clearly demonstrated that the majority of prey groups were generally strongly identified as themselves in the QFASA modeling. Nevertheless, there were some especially problematic prey groups, which must be evaluated in modeled diets. And as discussed before, minor occurrences of prey in modeled diets could represent real intake or noise, and thus should be interpreted with caution.

The results of the QFASA modeling of actual monk seals reveal several important things. Overall, monk seals appear to depend in large part on deep-slope species, which is consistent with recent results coming from dive and location analyses, as well as direct visual evidence from deployment of CRITTERCAM[TM] (Parrish et al., 2000). However, there was substantial variation in diet among individuals, demographic groups (especially between juveniles and adults/subadults) and locations, as evidenced by the complementary results of both discriminant analyses and QFASA diet estimates. Additionally, in repeat-sampled individuals, estimated diets tended to be similar within individuals; the largest differences that were found within an individual were particularly when a seal was sampled as a juvenile and then later as a subadult (Fig. 45).

In terms of diet estimates, there remain some issues that require caution in interpretation. It is interesting that snappers were consistently underestimated in most pseudo seal diet simulations, but that deepwater snappers appeared abundantly and consistently in estimated diets. Although there may be some overlap in the three deepwater snappers (e.g., Fig. 39), the prey on prey models (Fig. 38) suggest they are well-differentiated. Thus, it may be that it should at least be acknowledged that diet estimates may represent some overlap among these deepwater snappers. The absence of some species in diets may be puzzling: although lobsters, eels and wrasses were well-simulated and well-differentiated, other than moray eels, they did not appear in estimated diets. It is possible that earlier diet estimates based on scats and spews (Goodman-Lowe et al., 1999; Goodman-Lowe, 1998), which identified these as diet items, were entirely biased to nearshore feeding and resistant hard parts (or that spews of eels represent "non-intake" of those prey), rather than the main prey that dominate the diet from primarily deepwater feeding. Conversely, it is possible that these prey could be mistaken for other prey that were identified. Wrasses were correctly estimated at > 80% from the prey on prey models, and not mistaken for any other single major prey group (Fig. 38). However, when wrasses were modeled on other prey, without them being in the prey base at all (i.e., by nature of the model, they must go to other prey), they were indeed identified as a mixture of other prey, but none of which appeared in estimated monk seal diets in any case. Although prey such as box.crab and lobster were well-simulated in pseudo seal diets and well-differentiated in prey on prey models, evidence does suggest that overlap in crustaceans can occur, such that lobster could be mistaken for box.crab; however, the overall abundance of lobster in the ecosystem remains low and intakes could simply be masked by major diet items.

Thus, some questions remain in interpreting the findings of these analyses. We completed all analyses possible with the resources available, but there remain questions that could be addressed in the future. We are confident that we have sampled most of the important species to monk seals, but this has not been an easy task – for some species, collection of even a few individuals has required incredible effort. Nevertheless, some species were not able to be obtained at all. For

instance, one of the most abundant slope species is a small fish in the Serranidae family, *Symphysanodon maunaloae*, that comprises much of the diet of the deep slope snappers (Randall, 2007) and could not be obtained. Thus, whether its signature overlaps with that of other deepwater snappers is unknown. An investigation of how FA signatures of prey vary among habitats, across food webs and trophic levels, and within multispecies foraging aggregations would provide important insight into how we model seals and whether we should use prey within only certain regions. Further work on QFASA simulations could provide insight into the mathematical issues that arise when too many species are specified in the pseudo diet and how this relates to the actual modeling of the predator. Diet estimates could be further elucidated through additional series of analyses, such as sequential leave-one out modeling, or further analyses of prey groups and grouping procedures. These are currently beyond the scope of the current work, but could be considered.

Future Work

While this work has provided a very useful tool for monk seal diet studies, more work needs to be done on enhancing and refining the diet model. The first priority is the refinement of the prey database. The results of this study and fecal analysis can better inform us on species that can be removed from the prey database to allow for the addition and regrouping of others. Additional effort needs to be given to the collection and incorporation of deepwater slope species, including a number of bottomfish, into the prey library. The PIFSC will develop a sample collection strategy based on what prey are determined to be needed for the library. Collection of prey from the Papahānaumokuākea Marine National Monument may be difficult because of permit issues so other sites may need to be considered for collection. At this time it is difficult to know what type of bias this could bring to the model because of the geographic variability of fatty acid profiles. It would be useful to increase the sample size of seals sampled in the MHI. The QFASA model would also likely benefit by conducting a longer term captive study on Hawaiian monk seals. Ongoing QFASA work with other species will also provide very useful information pertinent to monk seal diet analyses.

ACKNOWLEDGMENTS

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Appendix A. Prey collection database for the Northwestern and Main Hawaiian Islands. Species and common names are listed, along with general group (note, this "group" is not the same as the 40 species groupings used in the current analyses, Table 2), and samples sizes by collection location.

Species Name	Common Name	Family	Group	CSM	Main	Niihau	Nihoa	Necker	FFS	GP	MR	Laysan	Midway	Smt 11	Unk	Total
Enchrasicholina purpurea	Hawaiian anchovy	Engraulidae	anchovies		20											**20**
Centropyge potteri	Potter's angelfish	Pomacanthidae	angelfishes						19		1					**20**
Antigonia capros	boar fish	Pentacerotidae	armourheads & boarfishes		10											**10**
Antigonia eos	boar fish_2	Pentacerotidae	armourheads & boarfishes		10											**10**
Pseudopentaceros wheeleri	armorhead	Pentacerotidae	armourheads & boarfishes											20		**20**
Polymixia berndti	Berndt's beard fish	Polymixiidae	beardfish						15	5						**20**
Priacanthus alalaua	Forskal's big-eye fish	Priacanthidae	bigeyes		11			7		1	1					**20**
Priacanthus boops	Schneider's bigeye	Priacanthidae	bigeyes					1								**1**
Priacanthus meeki	Hawaiian bigeye	Priacanthidae	bigeyes						20							**20**
Exalias brevis	shortbodied blenny	Blenniidae	blennies					1	7							**8**
Brotula multibarbata	large-eye brotula	Ophidiidae	brotulas & cusk eels						9							**9**
Ophidion muraenolepis	black edged cusk eel	Ophidiidae	brotulas & cusk eels						11	6	3					**20**
Chaetodon fremblii	bluestripe butterflyfish	Chaetodontidae	butterflyfishes					12	7		3					**22**
Chaetodon kleinii	Klein's butterflyfish	Chaetodontidae	butterflyfishes					1								**1**
Chaetodon miliaris	milletseed butterflyfish	Chaetodontidae	butterflyfishes					18	9							**27**
Chaetodon multicinctus	multiband butterflyfish	Chaetodontidae	butterflyfishes						19							**19**
Chaetodon ornatissimus	ornate butterflyfish	Chaetodontidae	butterflyfishes						20							**20**
Chaetodon quadrimaculatus	four spot butterflyfish	Chaetodontidae	butterflyfishes						20							**20**
Forcipiger flavissimus	forcepfish	Chaetodontidae	butterflyfishes						26							**26**
Heniochus diphreutes	pennantfish	Chaetodontidae	butterflyfishes					12	12		12					**36**
Apogon maculiferus	spotted cardinalfish	Apogonidae	cardinalfishes					11	4		3					**18**
Apogon taeniopterus	bandfin cardinalfish	Apogonidae	cardinalfishes						1							**1**
Conger cinereus	moustache conger	Congridae	conger & garden eels					13	1		6					**20**
Ariosoma marginatum	big-eye conger	Congridae	conger & garden eels						11	13			2			**26**
Meadia abyssalis	Abyssal cut-throat eel	Synaphobranchid	cutthroat eels												19	**19**
Abudefduf abdominalis	Hawaiian sergeant	Pomacentridae	damselfishes						11				9			**20**
Abudefduf sordidus	blackspot sargeant	Pomacentridae	damselfishes					6	15							**21**
Abudefduf vaigiensis	Indo-Pacific sergeant	Pomacentridae	damselfishes						16							**16**
Chromis ovalis	oval chromis	Pomacentridae	damselfishes						17		4					**21**
Dascyllus albisella	Hawaiian dascyllus	Pomacentridae	damselfishes					3	14				3			**20**
Plectroglyphidodon imparipenni	brighteye damselfish	Pomacentridae	damselfishes						1							**1**
Plectroglyphidodon johnstoniani	blue-eye damselfish	Pomacentridae	damselfishes						16	1	1					**18**
Stegastes fasciolatus	Pacific gregory	Pomacentridae	damselfishes						15				6			**21**
Callionymus decoratus	longtail dragonet	Callionymidae	dragonets						1	19						**20**
Bembrops filifera	duck-billed bembropsid	Percophidae	duckbills		19											**19**
Cantherines verecundus	shy filefish	Monacanthidae	filefishes & leatherjackets					3	5							**8**
Pervagor spilosoma	fantail filefish	Monacanthidae	filefishes & leatherjackets					11	9		3					**23**
	unidentified filefish	Monacanthidae	filefishes & leatherjackets					13								**13**
Kuhlia sandvicensis	Hawaiian flagtail	Kuhliidae	flagtails						19							**19**
Cypselurus spilonotopterus	Bleeker's flying fish	Exocoetidae	flyingfishes					3								**3**
Mulloidichthys flavolineatus	yellowstripe goatfish	Mullidae	goatfishes						19				2			**21**
Mulloidichthys vanicolensis	yellowfin goatfish	Mullidae	goatfishes					1	19							**20**
Parupeneus bifasciatus	doublebar goatfish	Mullidae	goatfishes					3	16							**19**
Parupeneus chrysonemus	yellowbarbel goatfish	Mullidae	goatfishes							19						**19**
Parupeneus cyclostomus	blue goatfish	Mullidae	goatfishes						10							**10**
Parupeneus multifasciatus	manybar goatfish	Mullidae	goatfishes					11	9							**20**
Parupeneus pleurostigma	sidespot goatfish	Mullidae	goatfishes					12	2	1	3		1			**19**
Parupeneus porphyreus	white saddle goatfish	Mullidae	goatfishes					3								**3**
	unidentified goatfish	Mullidae	goatfishes						5							**5**

A-1

Species Name	Common Name	Family	Group	CSM	Main	Niihau	Nihoa	Necker	FFS	GP	MR	Laysan	Midway	Smt 11	Unk	Total
Epinephelus quernus	Hawaiian grouper	Serranidae	groupers, anthiases, etc					12		5	3					**20**
Pseudanthias thompsoni	Thompson's anthias	Serranidae	groupers, anthiases, etc					12		1	5	7				**25**
Cirrhitops fasciatus	rebarred hawkfish	Cirrhitidae	hawkfishes					10	10		2					**22**
Dactyoptena orientalis	helmut gurnard	Dactylopteridae	helmut gurnards						4	20						**24**
Decapterus macarellus	mackerel scad	Carangidae	jacks & trevallys		9				1							**10**
Selar crumenophthalmus	bigeye scad	Carangidae	jacks & trevallys		10											**10**
Seriola dumerili	amberjack	Carangidae	jacks & trevallys					8								**8**
Bothus mancus	flowery flounder	Bothidae	lefteye flounders						15					1		**16**
Bothus pantherinus	panther flounder	Bothidae	lefteye flounders						39	36						**75**
Bothus thompsoni	Thompson's flounder	Bothidae	lefteye flounders						19		6					**25**
Saurida flamma	orangemouth lizardfish	Synodontidae	lizardfishes						4							**4**
Saurida gracilis	slender lizardfish	Synodontidae	lizardfishes						1							**1**
	slender lizardfish?	Synodontidae	lizardfishes											1		**1**
Synodus binotatus	twospot lizardfish	Synodontidae	lizardfishes						4					3		**7**
Synodus dermatogenys	sand lizardfish	Synodontidae	lizardfishes						2							**2**
Synodus lobeli	Lobel's lizardfish	Synodontidae	lizardfishes						20							**20**
Synodus variegatus	reef lizardfish	Synodontidae	lizardfishes					2	14					3		**19**
Trachinocephalus myops	snakefish	Synodontidae	lizardfishes		3		2		7	8						**20**
Zanclus cornutus	moorish idol	Zanclidae	moorish idol						20							**20**
Gymnothorax albimarginatus	whitemargin moray	Muraenidae	morays					11			9					**20**
Gymnothorax berndti	Berndt's moray	Muraenidae	morays					8	2	9	1					**20**
Gymnothorax eurostus	stout moray	Muraenidae	morays					7	1		4			2		**14**
Gymnothorax flavimarginatus	yellowmargin moray	Muraenidae	morays						11		8					**19**
Gymnothorax melatremus	dwarf moray	Muraenidae	morays					9	4							**13**
Gymnothorax meleagris	whitemouth moray	Muraenidae	morays					10	7	1						**18**
Gymnothorax nudivomer	yellowmouth moray	Muraenidae	morays						1							**1**
Gymnothorax steindachneri	Steindachner's moray	Muraenidae	morays					12		6	7					**25**
Gymnothorax undulatus	undulated moray	Muraenidae	morays					5	5		9					**19**
	unidentified moray	Muraenidae	morays											1		**1**
Chlorurus perspicillatus	spectacled parrotfish	Scaridae	parrotfishes						22							**22**
Chlorurus sordidus	bullethead parrotfish	Scaridae	parrotfishes						19							**19**
Scarus dubius	regal parrotfish	Scaridae	parrotfishes						15					4		**19**
Scarus psittacus	palenose parrotfish	Scaridae	parrotfishes						11							**11**
Diodon hystrix	porcupinefish	Diodontidae	porcupinefishes					2	5							**7**
Canthigaster coronata	crown toby	Tetraodontidae	puffers					2	2	13	3					**20**
Canthigaster jactator	Hawaiian whitespotted toby	Tetraodontidae	puffers						10	7	7					**24**
Canthigaster rivulata	maze toby	Tetraodontidae	puffers					2	8	6						**16**
Lagocephalus hypselogenion	Bleeker's balloon fish	Tetraodontidae	puffers								6					**6**
Torquigner florealis	floral puffer	Tetraodontidae	puffers		6					1	10	3				**20**
Carcharrhinus amblyrhyncos	gray reef shark	Carcharhinidae	requiem sharks					4								**4**
Kyphosus bigibbus	gray chub	Kyphosidae	rudderfishes & sea chubs						19							**19**
Kyphosus vaigiensis	lowfin chub	Kyphosidae	rudderfishes & sea chubs						19							**19**
Parapercis schauinslandii	redspotted sandperch	Pinguipedidae	sandperches						1	17						**18**
Dendrochirus barberi	Barber's scorpionfish	Scorpaenidae	scorpionfishes					10	1		8					**19**
Iracundus signifer	decoy scorpionfish	Scorpaenidae	scorpionfishes						1	12						**13**
Sebastapistes ballieui	spotfin scorpionfish	Scorpaenidae	scorpionfishes						4		15					**19**
Samariscus corallinus	coralline-red flounder	Samaridae	slender flounders							14						**14**
Callechelys lutea	Freckled snake eel	Ophichthidae	snake eels & worm eels				5									**5**
Myrichthys magnificus	magnificent snake eel	Ophichthidae	snake eels & worm eels						9		1	2				**12**

Species Name	Common Name	Family	Group	CSM	Main	Niihau	Nihoa	Necker	FFS	GP	MR	Laysan	Midway	Smt 11	Unk	Total
Ophichthus kunaloa	snake eel	Ophichthidae	snake eels & worm eels	20												**20**
Aphareus furca	smalltooth jobfish	Lutjanidae	snappers				5		1	2						**8**
Aprion virescens	green jobfish	Lutjanidae	snappers						9	1	10					**20**
Etelis carbunculus	squirrelfish snapper	Lutjanidae	snappers						10	9	2					**21**
Etelis coruscans	longtail red snapper	Lutjanidae	snappers						10		6					**16**
Lutjanus kasmira	bluestripe snapper	Lutjanidae	snappers						15	7						**22**
Pristipomoides filamentosus	pink snapper	Lutjanidae	snappers						16	8						**24**
Pristipomoides sieboldi	Siebold's snapper	Lutjanidae	snappers						3		12					**15**
Pristipomoides zonatus	flower snapper	Lutjanidae	snappers						6		13					**19**
Myripristis amaena	brick soldierfish	Holocentridae	soldierfish& squirrelfish										3			**3**
Myripristis berndti	bigscale soldierfish	Holocentridae	soldierfish& squirrelfish						2	16	2					**20**
Myripristis chryseres	yellowfin soldierfish	Holocentridae	soldierfish& squirrelfish						2							**2**
Neoniphon sammara	spotfin squirrelfish	Holocentridae	soldierfish& squirrelfish							13				6		**19**
Sargocentron diadema	crown squirrelfish	Holocentridae	soldierfish& squirrelfish							1						**1**
Sargocentron xantherythrum	Hawaiian squirrellfish	Holocentridae	soldierfish& squirrelfish						10	1	6	4				**21**
Acanthurus achilles	achilles tang	Acanthuridae	surgeonfish & unicornfish							20						**20**
Acanthurus dussumieri	eyestripe surgeonfish	Acanthuridae	surgeonfish & unicornfish							16						**16**
Acanthurus leucopareius	whitebar surgeonfish	Acanthuridae	surgeonfish & unicornfish						9	13						**22**
Acanthurus nigroris	bluelined surgeonfish	Acanthuridae	surgeonfish & unicornfish							14				6		**20**
Acanthurus olivaceus	orangeband surgeonfish	Acanthuridae	surgeonfish & unicornfish						10	10						**20**
Acanthurus triostegus	convict tang	Acanthuridae	surgeonfish & unicornfish							15				5		**20**
Ctenochaetus strigosus	goldring surgeonfish	Acanthuridae	surgeonfish & unicornfish							16						**16**
Naso hexacanthus	sleek unicornfish	Acanthuridae	surgeonfish & unicornfish		10											**10**
Naso lituratus	orangespine unicornfish	Acanthuridae	surgeonfish & unicornfish							19						**19**
Naso unicornis	bluespine unicornfish	Acanthuridae	surgeonfish & unicornfish						1	18						**19**
Zebrasoma flavescens	yellow tang	Acanthuridae	surgeonfish & unicornfish							20						**20**
Malacanthus brevirostris	flagtail tilefish	Malacanthidae	tilefishes					3	11	2						**16**
Melichthys niger	black triggerfish	Balistidae	triggerfishes							20						**20**
Melichthys vidua	pinktail durgon	Balistidae	triggerfishes							8						**8**
Sufflamen bursa	lei triggerfish	Balistidae	triggerfishes							19						**19**
Xanthichthys mento	crosshatch triggerfish	Balistidae	triggerfishes						1							**1**
Aulostomus chinensis	trumpetfish	Aulostomidae	trumpetfishes						12	6		6				**24**
Lactoria fornasini	thornback cowfish	Ostraciidae	trunkfishes								7					**7**
Ostracion meleagris	spotted boxfish	Ostraciidae	trunkfishes						13	7						**20**
Thunnus albacares	yellowfin tuna	Scombridae	tunas & mackerels		3											**3**
Anampses cuvier	pearl wrasse	Labridae	wrasses							16			4			**20**
Bodianus bilunulatus	Hawaiian hogfish	Labridae	wrasses						6	14						**20**
Cirrilabrus jordani	flame wrasse	Labridae	wrasses								11					**11**
Coris ballieui	lined coris	Labridae	wrasses				9	1	6							**16**
Coris flavovittata	yellowstriped coris	Labridae	wrasses							20						**20**
Coris venusta	elegant coris	Labridae	wrasses							17			3			**20**
Cymolutes lecluse	Hawaiian knifefish	Labridae	wrasses				2			10			11			**23**
Gomphosus varius	bird wrasse	Labridae	wrasses							7						**7**
Inistius pavo	peacock razorfish	Labridae	wrasses				4	4		2			5			**15**
Inistius umbrilatus	blackside razorfish	Labridae	wrasses				9	9		1						**19**
Macropharyngodon geoffroy	shortnose wrasse	Labridae	wrasses							20						**20**
Oxycheilinus unifasciatus	ringtail wrasse	Labridae	wrasses							19						**19**
Psuedocheilinus octotaeni	eightstripe wrasse	Labridae	wrasses							20						**20**
Stethojulis balteata	belted wrasse	Labridae	wrasses							18						**18**

Species Name	Common Name	Family	Group	CSM	Main	Niihau	Nihoa	Necker	FFS	GP	MR	Laysan	Midway	Smt 11	Unk	Total
Thalassoma ballieui	blacktail wrasse	Labridae	wrasses					3	14		2		1			**20**
Thalassoma duperry	saddle wrasse	Labridae	wrasses						18				2			**20**
Thalassoma purpureum	surge wrasse	Labridae	wrasses										2			**2**
Octopus cyanea	Hawaiian day octopus	Octopodidae	octopus					3	7		3					**13**
Octopus ornatus	ornate octopus	Octopodidae	octopus					2	2							**4**
Octopus sp.	unidentified octopus	Octopodidae	octopus		1			1	15	5	1					**23**
Stenoteuthis oualaniensis	neon flying squid	Ommastrephidae	squid		2			1							12	**15**
Heterocarpus ensifer	two-spined shrimp	Pandalidae	shrimp					11			20					**31**
Heterocarpus laevigatus	red-tipped shrimp	Pandalidae	shrimp					19		20						**39**
Calappa bicornis	two-horned box crab	Calappidae	box crab					10			13					**23**
Calappa calappa	smooth box crab	Calappidae	box crab					16	8	12						**36**
Calappa gallus	lumpy box crab	Calappidae	box crab						11							**11**
Ranina ranina	Kona crab	Raninidae	frog crab					1		10	10					**21**
Dardanus brachyops	short-eyed hermit crab	Diogenidae	hermit crab					7	5	10	7					**29**
Dardanus gemmatus	gemmate hermit crab	Diogenidae	hermit crab					17	7	8						**32**
Dardanus megistos	white-spotted hermit crab	Diogenidae	hermit crab					1	8		11					**20**
Dardanus sp.	unidentified hermit crab	Diogenidae	hermit crab					1								**1**
	purple leg hermit crab	Unknown	hermit crab						1							**1**
Homola ikedae	Ikedae homolid crab	Homolidae	homolid crab								2					**2**
Paramola alcocki	Alcock's homolid crab	Homolidae	homolid crab								2					**2**
Paramola japonica	Japanese homolid crab	Homolidae	homolid crab					3		7	14					**24**
Cryptodormis tidens	cryptic sponge crab	Dromiidae	sponge crab					2								**2**
Dromidiopsis dormia	sleepy sponge crab	Dromiidae	sponge crab					8		7	3					**18**
Charybdis hawaiiensis	Hawaiian swimming crab	Portunidae	true crab					9	5	10	5					**29**
Charybdis paucidentatus	red swimming crab	Portunidae	true crab					2			11					**13**
Cyrtomaia smithi	Smith's crab	Portunidae	true crab					6								**6**
Lupocyclus quinquedentatus	five-tooth swimming crab	Portunidae	true crab					2		7	6					**15**
Parthenope contrarius	elbow crab	Parthenopidae	true crab					8	10	2	1					**21**
Portunus sanguinolentus	white crab	Portunidae	true crab		10											**10**
Randallia distincta			true crab								4					**4**
Carpilius convexus	convex pebble crab	Xanthidae	xanthid crab					8	9		4					**21**
Carpilius maculatus	7-11 crab	Xanthidae	xanthid crab						3		9					**12**
Carpilius sp.	unidentified crab	Xanthidae	xanthid crab					2								**2**
Parabacus antarticus	chinese slipper lobster	Scyllaridae	slipper lobster					10	10		10					**30**
Scyllarides haanii	ridgeback slipper lobster	Scyllaridae	slipper lobster					10	6	10			4			**30**
Scyllarides squammosus	common slipper lobster	Scyllaridae	slipper lobster					31	12	10	8		11			**72**
Panulirus marginatus	spiny lobster	Palinuridae	spiny lobster		5			23	6	10			16			**60**
Panulirus marginatus	spiny lobster - tails only	Palinuridae	spiny lobster								11					**11**
Panulirus pencillatus	green spiny lobster	Palinuridae	spiny lobster					1	8							**9**
				20	**129**	**32**	**27**	**651**	**1381**	**406**	**309**	**7**	**117**	**39**	**12**	**3130**

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Appendix B.. Fat content (% fat) of the 178 fish and invertebrate prey species collected (n=3084) in the Hawaiian Islands, including percent lipid data for 46 unidentified specimens (n = 3130).

Species Name	Common Name	n	% Fat
Abudefduf abdominalis	Hawaiian sergeant	20	2.03 ± 0.84
Abudefduf sordidus	blackspot sargeant	21	2.44 ± 1.13
Abudefduf vaigiensis	Indo-Pacific sergeant	16	3.42 ± 2.33
Acanthurus achilles	achilles tang	20	4.41 ± 2.88
Acanthurus dussumieri	eyestripe surgeonfish	16	2.19 ± 0.77
Acanthurus leucopareius	whitebar surgeonfish	22	3.61 ± 1.91
Acanthurus nigroris	bluelined surgeonfish	20	4.22 ± 1.22
Acanthurus olivaceus	orangeband surgeonfish	20	2.98 ± 2.16
Acanthurus triostegus	convict tang	20	2.35 ± 1.70
Anampses cuvier	pearl wrasse	20	3.39 ± 1.47
Antigonia capros	boar fish	10	1.37 ± 0.37
Antigonia eos	boar fish_2	10	2.37 ± 0.81
Aphareus furca	smalltooth jobfish	8	1.97 ± 1.65
Apogon maculiferus	spotted cardinalfish	18	2.19 ± 0.77
Apogon taeniopterus	bandfin cardinalfish	1	0.84
Aprion virescens	green jobfish	20	1.20 ± 0.91
Ariosoma marginatum	big-eye conger	26	1.65 ± 0.47
Aulostomus chinensis	trumpetfish	24	2.41 ± 1.21
Bembrops filifera	duck-billed bembropsid	19	1.83 ± 1.40
Bodianus bilunulatus	Hawaiian hogfish	20	1.49 ± 0.55
Bothus mancus	flowery flounder	16	1.07 ± 0.28
Bothus pantherinus	panther flounder	75	2.11 ± 0.76
Bothus thompsoni	Thompson's flounder	25	2.02 ± 0.95
Brotula multibarbata	large-eye brotula	9	1.54 ± 0.50
Calappa bicornis	two-horned box crab	23	0.53 ± 0.35
Calappa calappa	smooth box crab	36	0.81 ± 0.48
Calappa gallus	lumpy box crab	11	0.64 ± 0.28
Callechelys lutea	Freckled snake eel	5	2.08 ± 0.18
Callionymus decoratus	longtail dragonet	20	1.19 ± 0.16
Cantherines verecundus	shy filefish	8	3.86 ± 1.82
Canthigaster coronata	crown toby	20	1.48 ± 0.65
Canthigaster jactator	Hawaiian whitespotted toby	24	1.59 ± 0.69
Canthigaster rivulata	maze toby	16	0.97 ± 0.73
Carcharhinus amblyrhyncos	gray reef shark	4	10.41 ± 2.36
Carpilius convexus	convex pebble crab	21	0.95 ± 0.48
Carpilius maculatus	7-11 crab	12	0.66 ± 0.37
Centropyge potteri	Potter's angelfish	20	4.30 ± 1.14
Chaetodon fremblii	bluestripe butterflyfish	22	4.35 ± 2.28
Chaetodon kleinii	Klein's butterflyfish	1	3.97
Chaetodon miliaris	milletseed butterflyfish	27	3.06 ± 1.87
Chaetodon multicinctus	multiband butterflyfish	19	3.41 ± 1.70
Chaetodon ornatissimus	ornate butterflyfish	20	3.38 ± 2.05
Chaetodon quadrimaculatus	four spot butterflyfish	20	4.67 ± 2.72
Charybdis hawaiensis	Hawaiian swimming crab	29	0.75 ± 0.40
Charybdis paucidentatus	red swimming crab	13	0.88 ± 0.35
Chlorurus perspicillatus	spectacled parrotfish	22	1.75 ± 1.22
Chlorurus sordidus	bullethead parrotfish	19	2.10 ± 0.79
Chromis ovalis	oval chromis	21	4.56 ± 2.08
Cirrhitops fasciatus	rebarred hawkfish	22	1.82 ± 0.77
Cirrilabrus jordani	flame wrasse	11	2.69 ± 0.39
Conger cinereus	moustache conger	20	2.04 ± 1.05
Coris ballieui	lined coris	16	1.84 ± 0.99
Coris flavovittata	yellowstriped coris	20	1.10 ± 0.39
Coris venusta	elegant coris	20	2.22 ± 0.80
Cryptodromis tridens	cryptic sponge crab	2	1.41 ± 0.27
Ctenochaetus strigosus	goldring surgeonfish	16	6.66 ± 4.11
Cymolutes lecluse	Hawaiian knifefish	23	2.14 ± 0.85
Cypselurus spilonotopterus	Bleeker's flying fish	3	2.74 ± 0.47
Cyrtomaia smithi	Smith's crab	6	0.51 ± 0.14
Dactyloptena orientalis	helmut gurnard	24	1.90 ± 0.71
Dardanus brachyops	short-eyed hermit crab	29	1.50 ± 0.64
Dardanus gemmatus	gemmate hermit crab	32	1.63 ± 0.68

Species Name	Common Name	n	% Fat
Dardanus megistos	white-spotted hermit crab	20	1.25 ± 0.50
Dascyllus albisella	Hawaiian dascyllus	20	3.29 ± 2.55
Decapterus macarellus	mackerel scad	10	3.40 ± 1.26
Dendrochirus barberi	Barber's scorpionfish	19	1.52 ± 0.61
Diodon hystrix	porcupinefish	7	2.44 ± 1.51
Dromidiopsis dormia	sleepy sponge crab	18	1.56 ± 0.77
Encrasicholina purpurea	Hawaiian anchovy	20	0.91 ± 0.22
Epinephelus quernus	Hawaiian grouper	20	2.66 ± 1.50
Etelis carbunculus	squirrelfish snapper	21	2.08 ± 0.74
Etelis coruscans	longtail red snapper	16	2.15 ± 1.13
Exallias brevis	shortbodied blenny	8	1.42 ± 0.35
Forcipiger flavissimus	forcepfish	26	3.62 ± 2.06
Gomphosus varius	bird wrasse	7	2.95 ± 1.89
Gymnothorax albimarginatus	whitemargin moray	20	0.84 ± 0.41
Gymnothorax berndti	Berndt's moray	20	1.41 ± 0.74
Gymnothorax eurostus	stout moray	14	1.42 ± 0.81
Gymnothorax flavimarginatus	yellowmargin moray	19	1.67 ± 1.03
Gymnothorax melatremus	dwarf moray	13	1.40 ± 0.84
Gymnothorax meleagris	whitemouth moray	18	1.50 ± 0.80
Gymnothorax nudivomer	yellowmouth moray	1	0.74
Gymnothorax steindachneri	Steindachner's moray	25	0.85 ± 0.37
Gymnothorax undulatus	undulated moray	19	1.83 ± 1.47
Heniochus diphreutes	pennantfish	36	3.94 ± 1.66
Heterocarpus ensifer	two-spined shrimp	31	2.57 ± 0.94
Heterocarpus laevigatus	red-tipped shrimp	39	1.93 ± 0.74
Homola ikedae	Ikedae homolid crab	2	1.24 ± 0.31
Iniistius pavo	peacock razorfish	15	1.81 ± 0.89
Iniistius umbrilatus	blackside razorfish	19	2.59 ± 0.93
Iracundus signifer	decoy scorpionfish	13	2.21 ± 0.71
Kuhlia sandvicensis	Hawaiian flagtail	19	6.96 ± 3.95
Kyphosus bigibbus	gray chub	19	1.68 ± 1.15
Kyphosus vaigiensis	lowfin chub	19	1.43 ± 0.71
Lactoria fornasini	thornback cowfish	7	3.88 ± 1.82
Lagocephalus hypselogenion	Bleeker's balloon fish	6	1.22 ± 0.48
Lupocyclus quinquedentatus	five-tooth swimming crab	15	1.67 ± 1.26
Lutjanus kasmira	bluestripe snapper	22	2.68 ± 1.04
Macropharyngodon geoffroy	shortnose wrasse	20	3.07 ± 1.17
Malacanthus brevirostris	flagtail tilefish	16	1.55 ± 0.51
Meadia abyssalis	Abyssal cut-throat eel	19	2.64 ± 0.97
Melichthys niger	black triggerfish	20	1.61 ± 1.43
Melichthys vidua	pinktail durgon	8	1.38 ± 0.76
Mulloidichthys flavolineatus	yellowstripe goatfish	21	1.86 ± 1.10
Mulloidichthys vanicolensis	yellowfin goatfish	20	2.31 ± 1.21
Myrichthys magnificus	magnificent snake eel	12	1.91 ± 1.48
Myripristis amaena	brick soldierfish	3	3.98 ± 0.71
Myripristis berndti	bigscale soldierfish	20	3.39 ± 1.74
Myripristis chryseres	yellowfin soldierfish	2	1.61 ± 0.40
Naso hexacanthus	sleek unicornfish	10	3.77 ± 1.27
Naso lituratus	orangespine unicornfish	19	7.44 ± 6.11
Naso unicornis	bluespine unicornfish	19	4.29 ± 3.31
Neoniphon sammara	spotfin squirrelfish	19	2.36 ± 2.41
Octopus cyanea	Hawaiian day octopus	13	1.12 ± 0.43
Octopus ornatus	ornate octopus	4	0.66 ± 0.39
Ophichthus kunaloa	snake eel	20	2.37 ± 0.80
Ophidion muraenolepis	black edged cusk eel	20	1.74 ± 0.71
Ostracion meleagris	spotted boxfish	20	3.46 ± 1.96
Oxycheilinus unifasciatus	ringtail wrasse	19	1.21 ± 0.57
Panulirus marginatus	spiny lobster	71	1.17 ± 0.46
Panulirus penicillatus	green spiny lobster	9	1.14 ± 0.51
Parabacus antarcticus	chinese slipper lobster	30	0.83 ± 0.30
Paramola alcocki	Alcock's homolid crab	2	0.59 ± 0.20
Paramola japonica	Japanese homolid crab	24	1.56 ± 0.90
Parapercis schauinslandii	redspotted sandperch	18	1.94 ± 0.79
Parthenope contrarius	elbow crab	21	0.60 ± 0.40
Parupeneus chrysonemus	doublebar goatfish	19	3.40 ± 1.16

Species Name	Common Name	n	% Fat
Parupeneus cyclostomus	yellowbarbel goatfish	10	1.72 ± 1.17
Parupeneus insularis	blue goatfish	19	1.40 ± 1.23
Parupeneus multifasciatus	manybar goatfish	20	1.51 ± 1.18
Parupeneus pleurostigma	sidespot goatfish	19	2.69 ± 1.77
Parupeneus porphyreus	white saddle goatfish	3	1.55 ± 1.34
Pervagor spilosoma	fantail filefish	23	3.36 ± 1.57
Plectroglyphidodon imparipennis	brighteye damselfish	1	2.52
Plectroglyphidodon johnstonianus	blue-eye damselfish	18	5.44 ± 2.03
Polymixia berndti	Berndt's beard fish	20	2.00 ± 0.90
Portunus sanguinolentus	white crab	10	1.17 ± 0.35
Priacanthus alalaua	Forskal's big-eye fish	20	2.84 ± 1.91
Priacanthus boops	Schneider's bigeye	1	0.68
Priacanthus meeki	Hawaiian bigeye	20	1.67 ± 1.21
Pristipomoides filamentosus	pink snapper	24	2.01 ± 2.10
Pristipomoides sieboldi	Siebold's snapper	15	2.57 ± 1.33
Pristipomoides zonatus	flower snapper	19	2.28 ± 0.99
Pseudanthias thompsoni	Thompson's anthias	25	3.02 ± 1.06
Pseudopentaceros wheeleri	armorhead	20	27.13 ± 4.62
Psuedochelinus octotaeni	eightstripe wrasse	20	3.36 ± 2.45
Randallia distincta		4	0.81 ± 0.22
Ranina ranina	Kona crab	21	0.93 ± 0.37
Samariscus corallinus	coralline-red flounder	14	1.37 ± 0.36
Sargocentron diadema	crown squirrelfish	1	0.75
Sargocentron xantherythrum	Hawaiian squirrelfish	21	4.06 ± 3.24
Saurida flemma	orangemouth lizardfish	4	1.19 ± 0.32
Saurida gracilis	slender lizardfish	1	0.69
Saurida gracilis?	slender lizardfish	1	0.88
Scarus dubius	regal parrotfish	19	4.44 ± 2.16
Scarus psittacus	palenose parrotfish	11	1.59 ± 0.62
Scyllarides haanii	ridgeback slipper lobster	30	0.99 ± 0.58
Scyllarides squammosus	common slipper lobster	72	0.91 ± 0.42
Sebastapistes ballieul	spotfin scorpionfish	19	1.19 ± 0.42
Selar crumenophthalmus	bigeye scad	10	1.58 ± 0.43
Seriola dumerili	amberjack	8	2.25 ± 1.94
Stegastes fasciolatus	Pacific gregory	21	3.72 ± 2.28
Stethojulis balteata	belted wrasse	18	3.09 ± 1.24
Sthenoteuthis oualaniensis	neon flying squid	15	1.87 ± 0.36
Sufflamen bursa	lei triggerfish	19	3.53 ± 2.23
Synodus binotatus	twospot lizardfish	7	1.41 ± 0.77
Synodus dermatogenys	sand lizardfish	2	1.03 ± 0.01
Synodus lobeli	Lobel's lizardfish	20	1.26 ± 0.48
Synodus variegatus	reef lizardfish	19	0.95 ± 0.65
Thalassoma ballieul	blacktail wrasse	20	1.46 ± 0.75
Thalassoma duperry	saddle wrasse	20	1.56 ± 0.57
Thalassoma purpureum	surge wrasse	2	1.77 ± 0.48
Thunnus albacares	yellowfin tuna	3	0.90 ± 0.19
Torquigner florealis	floral puffer	20	2.23 ± 1.45
Trachinocephalus myops	snakefish	20	0.93 ± 0.27
Xanthichthys mento	crosshatch triggerfish	1	3.87
Zanclus cornutus	moorish idol	20	4.12 ± 2.69
Zebrasoma flavescens	yellow tang	20	6.35 ± 3.02
	Sub-Total	3084	

Species Name	Common Name	n	% Fat
Unidentified specimens			
Unidentified filefish		13	1.58 ± 0.40
Unidentified goatfish		5	2.0 ± 1.31
Unidentified moray		1	1.13
Unidentified octopus		23	1.56 ± 0.84
Unidentified hermit crab		2	1.63 ± 0.22
Unidentified crab (Carpilius sp)		2	0.98 ± 0.31
	Sub-Total	46	
Grand Total		**3130**	

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Appendix C. FA sets used for QFASA simulations and modeling.

Fatty Acids	Dietary	Extended Dietary	Modified 1 Extended Dietary	Modified 2 Extended Dietary	Monk Seal "MS"	Monk Seal minus "MS minus"
	simulations	*simulations*	*simulations*	*simulations*	*diet est.*	*diet est.*
12:0						
13:0						
Iso14						
14:0		√	√	√	√	√
14:1n-9						
14:1n-7						
14:1n-5						
Iso15						
Anti15						
15:0						
15:1n-8						
15:1n-6						
Iso16						
16:0		√	√	√	√	√
16:1n-11						
16:1n-9						
16:1n-7		√		√	√	√
7Me16:0						
16:1n-5						
16:2n-6	√	√				
Iso17						
16:2n-4	√	√	√	√	√	√
16:3n-6	√	√	√	√	√	√
17:0		√		√		
16:3n-4	√	√	√	√	√	√
17:1						
16:3n-1						
16:4n-1	√	√	√			
18:0		√	√	√	√	√
18:1n-13						
18:1n-11						
18:1n-9		√	√	√	√	√
18:1n-7		√	√	√	√	√
18:1n-5			√		√	√
18:2d511						
18:2n-7						
18:2n-6	√	√	√	√	√	√
18:2n-4	√	√	√	√	√	
18:3n-6	√	√	√	√	√	
18:3n-4	√	√	√	√	√	

18:3n-3	√	√	√	√	√	√
18:3n-1	√	√	√	√	√	
18:4n-3	√	√	√	√	√	√
18:4n-1	√	√				
20:0						
20:1n-11	√	√	√	√	√	√
20:1n-9	√	√	√	√	√	√
20:1n-7	√	√	√	√	√	√
20:2n-6	√	√	√	√	√	√
20:3n-6	√	√		√	√	
20:4n-6	√	√	√	√	√	√
20:3n-3	√	√	√	√	√	√
20:4n-3	√	√	√	√	√	√
20:5n-3	√	√	√	√	√	√
22:1n-11	√	√	√	√	√	√
22:1n-9	√	√	√	√	√	√
22:1n-7	√	√		√	√	√
22:2n-6	√	√				
21:5n-3	√	√	√	√	√	√
22:4n-6	√	√	√	√	√*	√*
22:5n-6	√	√	√	√	√	√
22:4n-3	√	√	√		√	
22:5n-3		√	√	√	√	√
22:6n-3	√	√	√	√	√	√
24:1n-9						
Total Number of Fatty Acids	31	39	33	34	35	29

The first two FA subsets were taken primarily from Iverson et al. (2004). Dietary: includes only those 33 FAs that could arise from dietary origin and are reliably identified; extended-dietary (39 FAs) includes all "dietary" FAs as well as additional FAs that could be biosynthesized by predators, but whose levels in a predator are also highly influenced by consumption of specific prey. A third set of FAs ('modified 1 extended dietary') for simulations was created largely following work on captive Steller sea lions and harbor seals (S. Iverson and D. Tollit, unpublished data), which had measured equally reliable and comparable calibration coefficient (CC) estimates to those of monk seals. The fourth set of FAs ('modified 2 extended dietary'), was constructed from the extended dietary list, where FAs with an average mean of less than 0.10% mass percent in NWHI prey, and FAs that were routinely zeros in many NWHI species, were removed from the list. The first 4 sets were used in prey simulations. Based on these results, monk seals were modeled on these in addition to two additional sets, the first of which was almost identical to that of both modified 1 and modified 2 sets, but replaced two FAs that were either more or less reliably measured in the NWHI ecosystem and monk seals, while the last set removed any FA that was measured at close to zero in captive or wild monk seals, regardless of whether it had a CC with little variability. *22:4n-6 is very rare in northern/temperate ecosystems and is measured at almost zero in the North Atlantic ecosystem – and was therefore extremely low in both Atlantic herring and the captive monk seals consuming it (Fig. 3). However, in the NWHI ecosystem, this FA is more abundant and an important dietary component. Therefore, NWHI monk seals were modeled using both the CC (~6.0) determined for this FA from the captive monk seal feeding study, as well as with a more conservative value (closer to 1) of 2.0.

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